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Sarcomatous Transformation of the Stroma of Mammary Carcinomas That Stimulated Fibroblastic Growth *in Vitro**

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(Received for publication October 10, 1944)

INTRODUCTION

Sarcomatous transformation of the stroma of transplantable mouse carcinomas was first described by Ehrlich and Apolant (5). The following year a case was reported by Loeb (13). Subsequently the phenomenon was observed to occur twice in a strain of mammary carcinoma maintained in these laboratories, and a preliminary note on the subject was published by Bashford, Murray, and Haaland (4). Later a detailed histological investigation was carried out by Haaland, whose paper (8) effectively dealt with criticisms that had been raised as to the reality of sarcomatous transformations. "All evidence," he wrote, "seems to speak for a gradual process by which apparently normal connective tissue cells evolve into sarcomatous elements, endowed with altered biological qualities." Since this was written other cases have been reported from time to time, but sarcomatous change has been regarded in the past as a "rare occurrence." Thus in a general review of the tumors studied in these laboratories up to 1911, Bashford (2) commented, "the carcinomata which induce sarcomatous transformation of their stroma are of rare occurrence." This conclusion was based on observations upon more than 650 primary tumors and 85 strains of propagated tumors of various histological types. Sarcomatous transformation was observed in only four strains. "In two the change supervened during transplantation. In the other two the change took place in the primarily affected animal."

Of the various suggestions advanced as to the cause of sarcomatous change it is of interest to recall that Apolant and Ehrlich (1) attributed it to "a stimulating influence proceeding from the carcinomatous cells, which in a certain phase of their development determines the sarcomatous transformation of the connective tissue scaffolding of the tumor." Now, 38 years after this was written, we have been able to adduce evidence that mammary carcinomas that had "a stimulating influence" upon fibroblasts in tissue cultures have undergone sarcomatous change. In our previous paper (15) we demonstrated stimulation of fibroblastic growth *in vitro* by carcinomas of high mammary cancer strain mice. The present communication is concerned with the sarcomatous changes occurring in those tumors that were maintained by transplantation. Contrary to the experience of the earlier investigators, who worked with hybrid mice of unknown genetic constitution, our experiments indicate that sarcomatous change is a common occurrence in mice of high mammary cancer strains.

It should be pointed out that the evidence for the occurrence of sarcomatous transformation has not been universally accepted as conclusive, based as it is on the histological interpretation of transitional stages. An alternative explanation negatives the idea of neoplastic changes in the stroma, and attributes what appear to be sarcoma cells to morphologically altered epithelial cells (spindle cell metaplasia). "Such transformations are relatively common in the early or advanced stages of many human tumors, and especially in recurrences after operation, and this fact establishes a probability that a similar change in mouse tumors has a similar significance." So wrote Ewing (6), who concluded: "Since such an interpretation is at least admissible, it may be urged that further evidence is required before the sarcomatous transformation of mouse carcinoma can be accepted as proved."

Our purpose in this paper is to furnish further evi-

* Because of the difficulties of international communication the authors have not read proof of this article.

** Laura de Saliceto Student of the University of London.

dence of a different kind, based upon the behavior in tissue cultures of tumors before and after undergoing sarcomatous transformation. It will be demonstrated that tumors originating after repeated transplantations of mammary carcinomas and presenting the histological structure of sarcomas, exhibit the growth characteristics *in vitro* of typical sarcomas. They are essentially similar to sarcomas induced by the subcutaneous injection of carcinogenic compounds, and differ profoundly from the mammary carcinomas in the stromas of which they originated.

EXPERIMENTS

Four different mammary carcinomas that arose spontaneously in mice of the Strong A strain, and 2 that originated in mice of the RIII strain of Dobrovolskaia-Zavadskaia, were transplanted serially by subcutaneous grafts so as to have available a supply of tumors for testing therapeutic substances. At each transplantation a fragment of tumor was fixed for histological examination, and tumors of different generations were also grown in tissue cultures. It was observed that both the histological sections and the morphology of the culture exhibited a progressive change from carcinoma to sarcoma. The histological changes occurring during sarcomatous transformation of the stroma of carcinomas were very fully described by Haaland (8). We are able to endorse his statements that "neither in the primary tumour nor in the earlier generations of the strains leading up to sarcoma development do we find any stroma elements with peculiar characters"; and that "there is a definite stage before the appearance of the sarcoma in which the stroma of the tumour has become more abundant and cellular." In addition to the 6 tumors that we have had under observation during the sarcomatous transformation, 5 others of high mammary cancer strains have begun to exhibit this presarcomatous change.

That the 6 tumors studied by us have definitely undergone sarcomatous transformation, and not merely a spindle cell metaplasia, is most lucidly demonstrated by the tissue culture experiments. There is universal agreement that carcinomas grow typically as flat sheets, or as pavement-like growths of closely adherent cells, while in sarcoma cultures the cells are spindle-shaped or polymorphous and separated to varying degrees. (Lambert and Hanes [10]; subsequent work reviewed by Fischer [7], Ludford [14], and Levi [11].) A 4 day old coverglass culture of a primary tumor of a Strong A mouse is shown in Fig. 1. The carcinoma cells spread themselves out with facility, so that the living culture presents the appearance of a rounded, translucent membrane. The lighter area between the explant and the periphery of the growth in the photograph is the result of partial digestion of the plasma medium. When the plasma is liquefied these mammary carcinoma cells will usually spread out on the surface of the coverglass in the liquefied areas. The very different type of growth of a tumor of the sixth generation approximately 6 months after the first transplantation is illustrated in Fig. 2. The culture medium was the same in both cases, but Fig. 2 represents a 5 day old culture. It is composed of spindle-shaped cells becoming progressively more separated as they migrate peripherally. Another very significant difference between the 2 cultures is the large number of cells of the monocyte-macrophage type seen in Fig. 2. It is, in fact, a typical sarcoma culture, and sections of the tumor from which this culture was prepared show it to be a spindle cell sarcoma.

Part of another 3 day old culture prepared from an eighth generation transplant of a different mammary carcinoma of a Strong A mouse is represented in Fig. 3. Here again is seen the typical sheet-like growth characteristic of carcinomas *in vitro*. The irregular form of the growth, with small isolated islands of cells, is the result of explanting numerous small fragments of tumor, some of which coalesced as they grew. This culture again contains very few fibroblasts or cells of the monocyte-macrophage type. It will be observed that after 8 transplantations, the eighth being approximately 7 months after the primary tumor was first transplanted, this tumor was still growing *in vitro* as a typical carcinoma.

A sarcomatous growth from a tumor of the 19th generation transplant is shown in Fig. 4. This is comprised of numerous separate large spindle-shaped to polymorphous cells, again with very considerable numbers of cells of the monocyte-macrophage type. These extend out into the medium far beyond the outermost of the sarcoma cells.

This particular tumor was unique amongst our 6 cases in that large atypical cells were first observed in cultures from a tumor of the 11th generation, approximately 9 months after the first transplantation. Since these cells exhibited the same cytological characters as have persisted throughout subsequent generations and now comprise the greater part of the tumor, we consider their appearance as indicatory of the sarcomatous transformation. But they did not rapidly overgrow and replace the carcinoma cells *in vivo*, which is what occurred with the other 5 tumors. Instead, they grew along with the carcinoma cells, constituting a carcinosarcoma. When small fragments of a tumor were explanted, 3 types of cultures resulted; (a) sheets of carcinoma cells, (b) growths of large spindle cells with innumerable monocytes and macrophages, and (c) mixtures of these two. Sometimes there was a sheet growth on one side of a culture and spindle cell

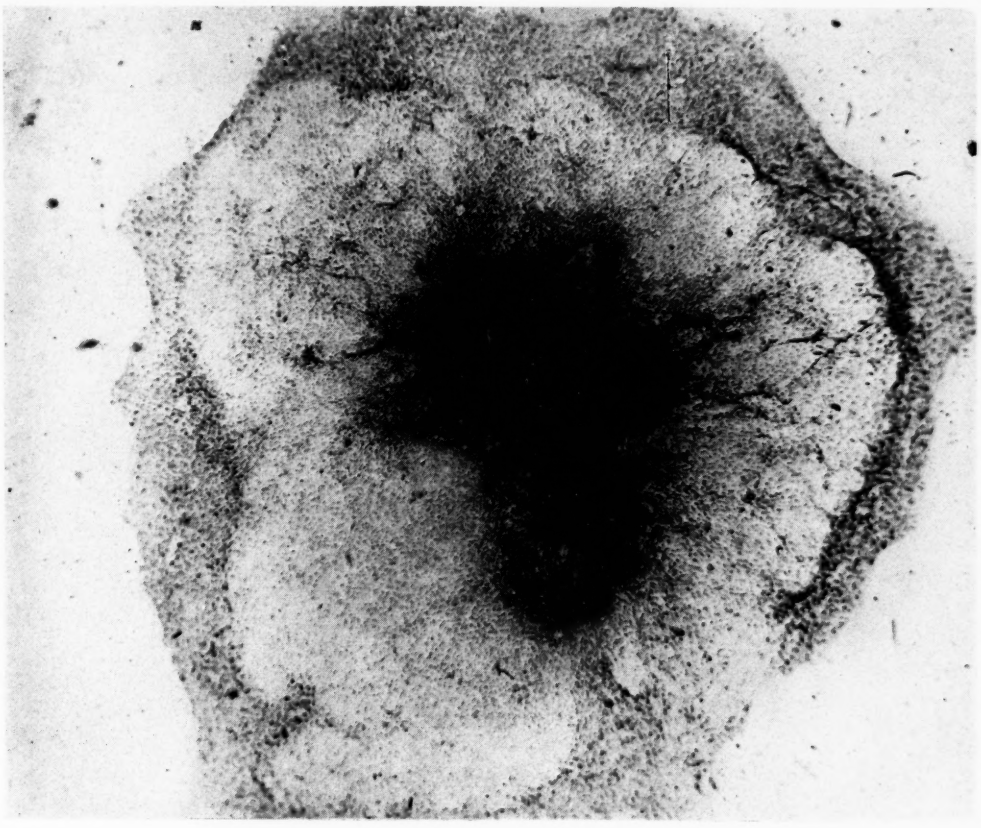

FIG. 1.—Four day old culture of a mammary carcinoma of a Strong A mouse (primary tumor).

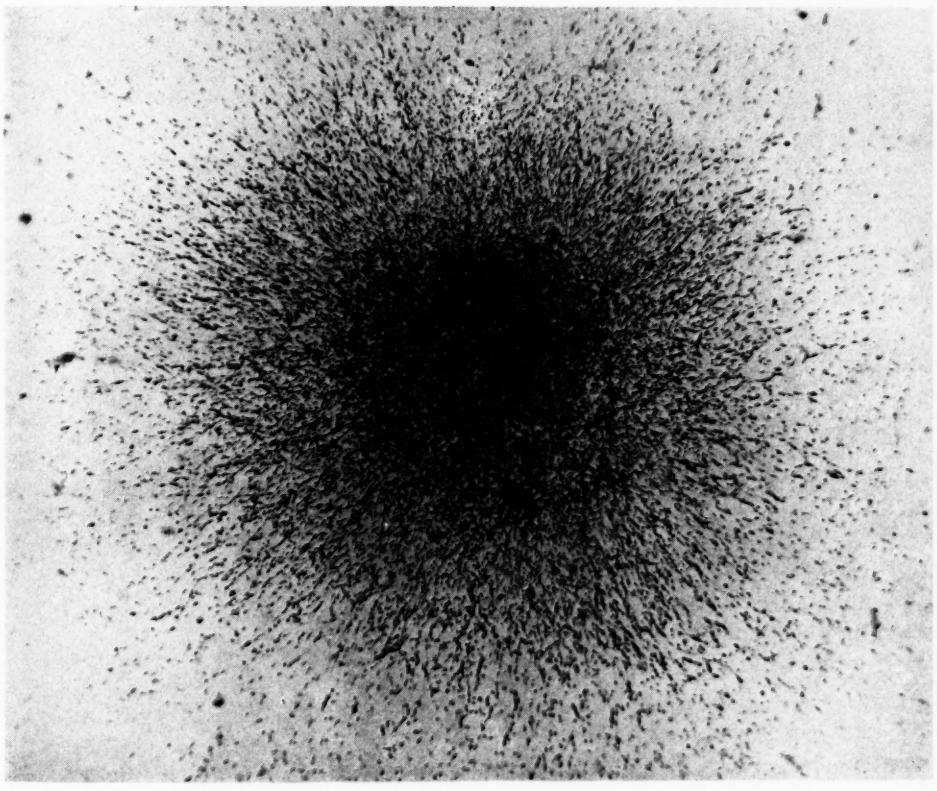

FIG. 2.—Five day old culture of the sarcoma that originated as a result of sarcomatous transformation of the stroma during transplantation of the mammary carcinoma shown in Fig. 1, at the same magnification.

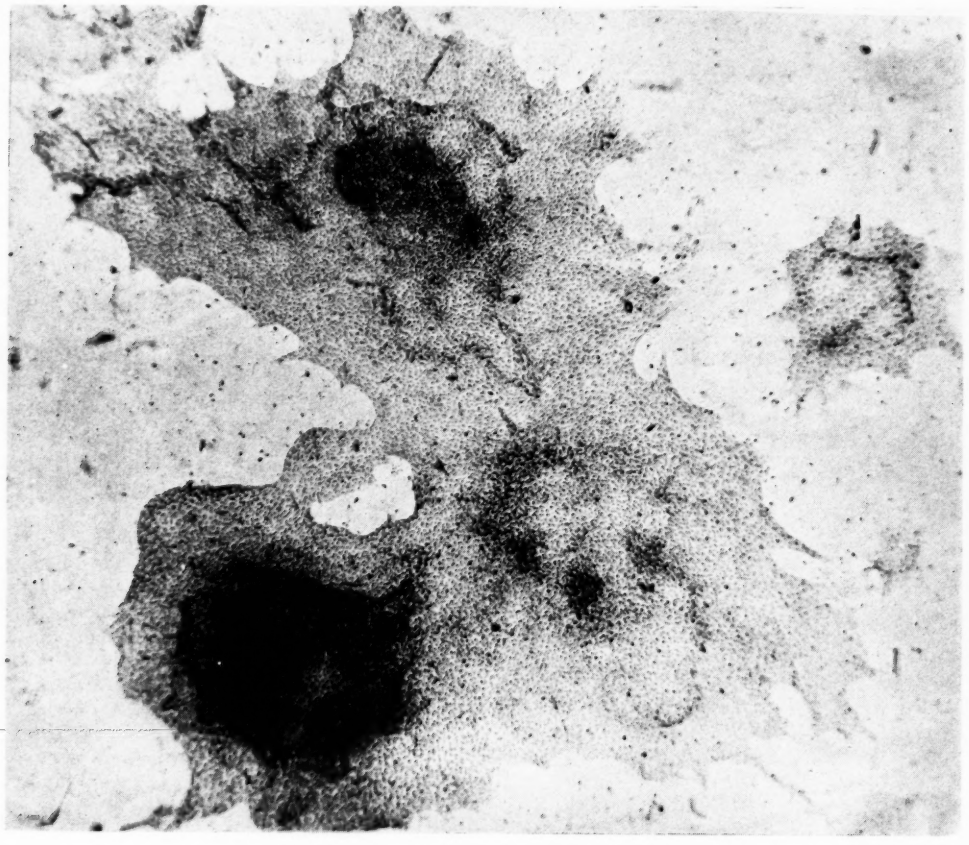

FIG. 3.—Three day old culture of another mammary carcinoma of a Strong A mouse (eighth generation transplant).

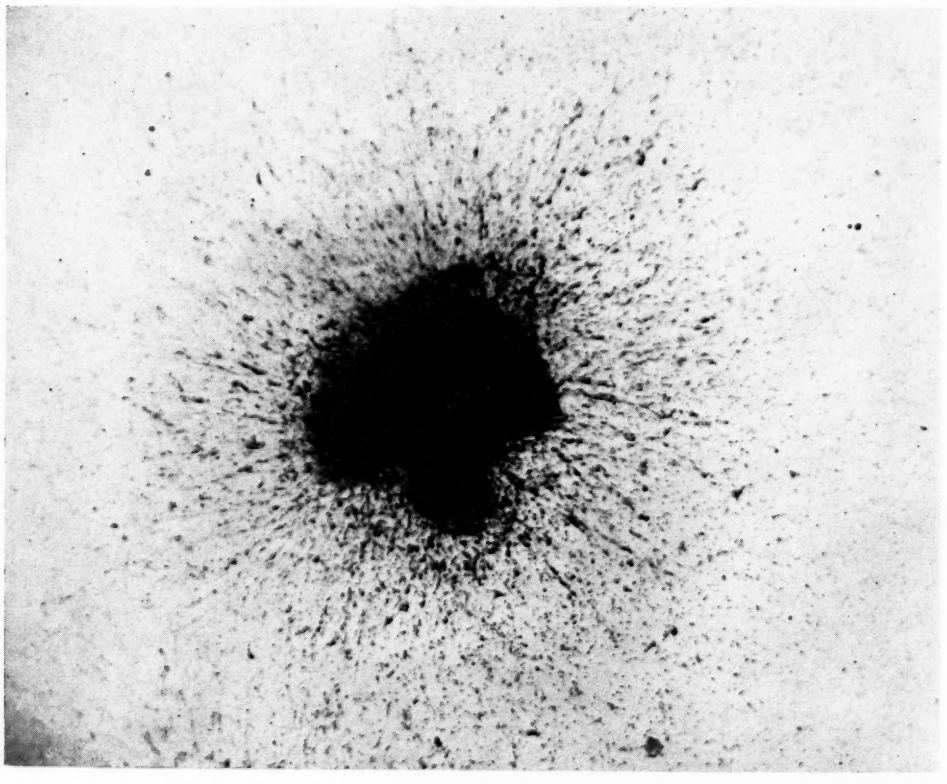

FIG. 4.—Three day old culture of the sarcoma resulting from sarcomatous transformation of the stroma of the mammary carcinoma illustrated in Fig. 3, at the same magnification.

growth on the other side. Most frequently there was a considerable outgrowth of sarcoma cells and monocytes and macrophages, and a residual compact growth of carcinoma cells within the explant. In the culture illustrated in Fig. 4 there are considerable numbers of carcinoma cells in the explant. Though there are well defined cytological differences between the carcinoma cells and the sarcoma cells, in addition to their shape and mode of growth, it is not intended to enter here into a discussion of the detailed cytology of these tumors; this will be reported in a later paper.

Sections of this tumor during its carcinosarcomatous phase exhibit large compact groups of carcinoma cells, in an extensive stroma consisting of hyperchromatic cells varying notably in size, with a considerable admixture of cells of the monocyte-macrophage type. Sections of the 20th generation still show areas of carcinoma and sarcoma, but suggest that the carcinomatous element will eventually be eliminated.

With the other 5 tumors the sarcomatous change was completed very rapidly; as far as we are able to determine, within a single generation, though our methods for distinguishing between fibroblasts, presarcomatous fibroblasts, and sarcoma cells lack the precision necessary for an unequivocal decision. Cultures prepared from tumors, the sections of which exhibited the presarcomatous changes of a more abundant and cellular stroma, were characterized by a considerable growth of fibroblasts with macrophages, usually extending outwards beyond the sheet growth of carcinoma cells. The fibroblasts that first appeared differed considerably from the sarcoma cells that grew ultimately. Although it is not always possible to distinguish with certainty whether any one cell is an abnormal fibroblast or a definite sarcoma cell, yet, as Lewis (12) has pointed out, sarcoma cells in general are characterized by an "increase in size of cell and nucleus, increase in density of cytoplasm, increase in number and decrease in size of the mitochondria, increase in the amount of nucleolar material, increase in thickness of the nuclear membrane and the granular condition of the nucleoplasm."

Were our evidence for sarcomatous transformation of these mammary carcinomas limited to differences in cellular morphology and in their growth patterns *in vitro* it would amount to little more than an amplification of the histopathological data. But we are able to add to it the demonstration of a difference in the biological properties of the cells that exhibit the different morphological features. In a previous paper (15), we adduced evidence that cultures of mammary carcinomas from high cancer strain mice stimulate the growth of fibroblasts, while sarcomas induced by carcinogenic hydrocarbons inhibit fibroblastic growth. We have employed the same technic to determine the influence on fibroblastic growth of these tumors when they are growing as typical carcinomas (Figs. 1 and 3) and as sarcomas (Figs. 2 and 4).

Mouse fibroblasts were grown between two explants of carcinoma and later, when the tumor exhibited the morphological indications of complete sarcomatous change, between two explants of this tumor. Growth of fibroblasts in the presence of the 2 tumors was compared with the growth of fibroblasts in the presence of other cultures of the same fibroblasts. The technic of cultivation and the method of measuring fibroblastic growth was the same as previously described. The carcinomas exhibited fibroblastic growth stimulation of the same order as described in our former paper. The tumors derived from their stromas, which were diagnosed histologically as sarcomas, inhibited fibroblastic growth to varying degrees, as had the sarcomas induced by carcinogenic hydrocarbons, with which our previous experiments were conducted. Figs. 5 and 6 demonstrate the difference in the growth of fibroblasts in the presence of a transplanted RIII carcinoma (Fig. 5) and of the spindle cell sarcoma to which it gave origin (Fig. 6). Both were photographed at the same magnification. Fig. 5 represents the best growth of fibroblasts obtained in this particular experiment after 7 days. Since fibroblastic growth in untreated cultures invariably ceases sooner in the presence of sarcoma than in the presence of carcinoma, the culture shown in Fig. 6 of the series, was fixed after 5 days, as it had begun to exhibit early indications of cellular degeneration. The 2 cultures are thus not strictly comparable, but in spite of the discrepancy in age of these 2 fibroblast cultures it is obvious that they have been subjected to different types of action, which have been determined by the different biological properties of the cells responsible.

DISCUSSION

As the foregoing observations indicate, tumors that from histological evidence are considered to have originated from fibroblasts of the stroma of mammary carcinomas are indeed true sarcomas. Their sarcomatous nature is indicated in cultures by their growth pattern and general cellular morphology, which resembles that of fibroblasts rather than of epithelial cells; by their high content of cells of the monocyte-macrophage type; and by their property of inhibiting the growth of fibroblasts. That the first 6 tumors with which we commenced this work should all have undergone sarcomatous transformation, apart from others now showing presarcomatous changes, implies that such transformations are a common occurrence in high cancer strains, at least in the Strong A and RIII strains. The selection of these tumors for transplantation in the first instance was purely arbitrary. They

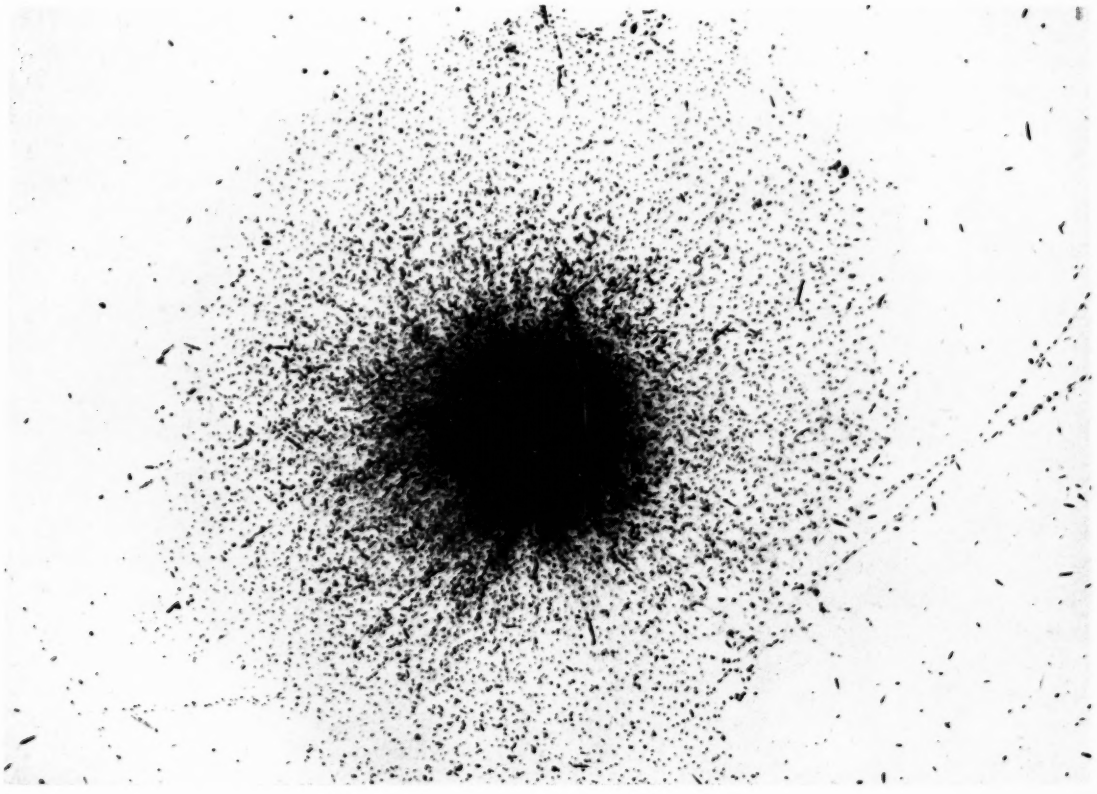

FIG. 5.—Culture of embryonic mouse fibroblasts grown between two explants of a mammary carcinoma from an RIII mouse (third generation transplant). Seven day old culture.

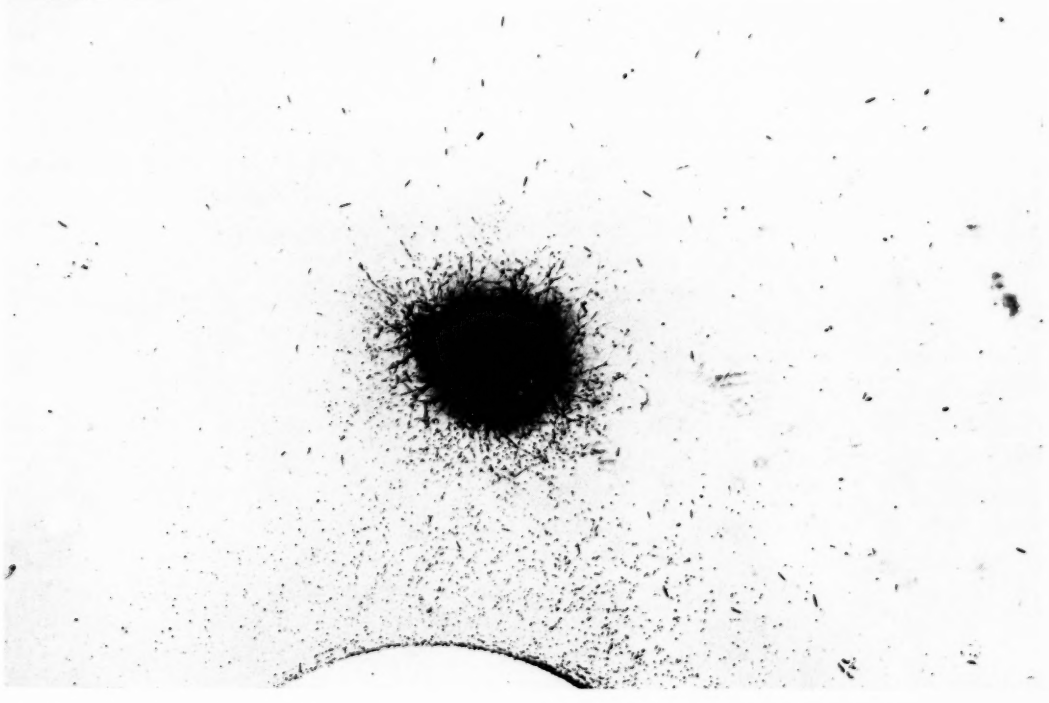

FIG. 6.—Culture of embryonic mouse fibroblasts grown between 2 explants of the sarcoma that originated by sarcomatous transformation of the stroma of the RIII tumor that stimulated the growth of fibroblasts illustrated in Fig. 5. Five day old culture, same magnification as Fig. 5.

were taken at random from our stocks of inbred mice as being of sufficient size to yield abundant tissue for transplantation into a large number of mice. The method of transplantation varied from time to time. When large numbers of mice were to be inoculated, tumors were minced to a pulp with scissors and injected with a syringe, but when transplantation was limited to a few mice, fragments of tissue, about 2 mm. in diameter, were inoculated with a trochar. We have no evidence that either method of transplantation specifically influenced the induction of the sarcomatous change.

In seeking an explanation for the frequency of the sarcomatous change certain factors demand special consideration. Particularly significant is the fact that all our mammary carcinomas that underwent this change had been found to be powerful stimulants of fibroblastic growth *in vitro,* as previously reported. This can mean only that something was liberated by the growing carcinoma cells that excited the fibroblasts to increased growth. We have no definite proof that a similar stimulation was operative *in vivo,* but its occurrence was indicated by the more abundant and cellular character of the stroma that preceded the sarcomatous changes. As was pointed out in our previous paper the carcinomas that stimulate fibroblastic growth most conspicuously are the mammary carcinomas of high cancer strain mice, and these are the tumors that have exhibited sarcomatous transformation of the stroma. In their etiology the "mammary tumor inciter" of Bittner is of fundamental importance, but we have as yet no evidence whether or not it is concerned in the sarcomatous transformation. Experiments directed towards elucidation of this aspect of the problem are still in progress.

That the special genetic constitution of the high mammary cancer strains may be an important factor seems highly probable, but here again our evidence is as yet equivocal and will be left for discussion to a later communication. Attention should be directed, however, to the difference between our transplantation experiments with inbred strains of mice and the work of the earlier investigators, who used mice of mixed genetic constitution. Thirty-nine years ago Bashford, Murray, and Cramer (3) published the first account of the "source of the constituent elements of new growths obtained by artificial propagation." They confirmed the earlier observations of Jensen (9), that the new tumor parenchyma is derived solely from that introduced, and demonstrated that the "stroma and vascular structures are merely a reaction on the part of the successive hosts, whereby the parenchyma is nourished and supported by an artificial circulation renewed from time to time." They described the stroma of carcinoma grafts as beginning to degenerate 24 hours after inoculation, as being "extremely degenerated in all its elements" after 3 days, and as reaching "the last stage of degeneration" after 4 days. In his study of sarcomatous transformation Haaland (8) was led to consider the possibility of fibroblasts of the stroma surviving transplantation, and their "survival after repeated subtransplantation into successive hosts" was suggested as a contributing factor to the induction of malignancy. He contributed corroborative evidence about which he wrote: "In examining early stages of tumours in this presarcomatous stage with abundant and cellular stroma, we found in single cases strong evidence of connective-tissue elements being transplantable, before any sarcomatous change shows itself histologically. We have seen the difficulties in the way of deciding when this transplantability of individual stroma elements has appeared for the first time, and the possibility remains that the transplantation of individual stroma elements may go further back than can be proved by our methods." It might be expected that when a tumor is transplanted into mice of the same genetic constitution, the likelihood of stroma elements surviving would be much enhanced, since the cells of the transplanted tumor stroma are then homozygous with those of the new hosts. We have investigated this possibility and, without entering into the details of our findings here, it is pertinent to point out that our evidence indicates that stromal cells from even the first generation transplant of a mammary carcinoma appear to survive when transplanted into a new host. Stromal cells at the periphery of such a graft do not undergo the early degenerative changes that Bashford, Murray, and Cramer described, but it is not possible to be absolutely certain that they survive to proliferate indefinitely, owing to the difficulty of distinguishing between these cells and others that invade the graft in bringing about the new vascularization.

Of the various factors, then, that might be responsible for the frequency of sarcomatous transformation during the transplantation of mammary carcinomas of high cancer strain mice, our present evidence emphasizes particularly the significance of the stimulation of fibroblastic growth by the carcinoma cells, and the greater survival of stromal cells when transplanted because they are homozygous with the cells of their new host.

SUMMARY

1. Sarcomatous transformation of the stroma is a common occurrence during the transplantation of mammary carcinomas of high cancer strain mice.

2. The histological evidence of sarcomatous change is confirmed by study of the growth characteristics of tumors *in vitro* before and after they have undergone transformation.

3. In tissue cultures mammary carcinomas exhibit the typical epithelial growth pattern, with few cells of the monocyte-macrophage type, and stimulate fibroblastic growth (Figs. 1, 3, and 5).

4. The sarcomatous nature of the transformed tumors is indicated by their growth pattern and general cellular morphology, resembling fibroblasts; by their high content of cells of the monocyte-macrophage type; and by their inhibiting fibroblastic growth (Figs. 2, 4, and 6).

5. Of the factors responsible for the frequency of sarcomatous change in the high mammary cancer strains, special significance is attributed to: (a) the considerable stimulation of fibroblastic growth by the carcinoma cells; and (b) stromal cells surviving transplantation because the cells of the graft are homozygous with those of the new host.

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9,10-Dimethyl-1,2-Benzanthracene as a Highly Potent Carcinogen for the Rabbit's Skin*

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(Received for publication October 24, 1944)

There are many types of investigation connected with the tumor problem for which a large laboratory animal such as the rabbit would be more useful than a small one such as the mouse. For problems connected with experimental carcinogenesis the limiting factor is, naturally, the availability of a suitable carcinogen.

Neither tar nor 3,4-benzpyrene can be considered as suitable for the rabbit: The objection to the former is that it is a complex mixture of varying composition, action, and toxicity; to the latter, that its action on the rabbit is very weak. As for the many other polycyclic hydrocarbons that are known to produce skin tumors in mice, few seem to have been tested on the skin of the rabbit (2).[1] Hence it is not possible to say from previous studies whether the rabbit can or cannot be extensively used for the detailed investigation of carcinogenesis.

It has been established that for mouse skin the most potent of the known agents is 9,10-dimethyl-1,2-benzanthracene (1, 3). It seemed desirable, therefore, to test this compound on the rabbit's skin also, and to compare the results with those obtained with tar and benzpyrene.

EXPERIMENTAL

The animals used for these experiments were young adult white rabbits, bred in this laboratory from a common stock and thus constituting a fairly homogeneous, though not genetically pure, strain. Their diet varied according to seasonal supplies, and included hay, oats, bread, cabbage, clover, mangolds, and carrots.

All solutions employed were kept in tightly-corked, dark bottles. They were applied to the skin with a Pasteur pipette, the amount deposited (about 8 drops) being just sufficient to cover most of the inner surface of the ear. All applications were made at half-weekly intervals.

RESULTS

9,10-Dimethyl-1,2-benzanthracene (1 per cent in benzene) was applied for 26 weeks to the right ears of 5 rabbits. Within a few seconds of the first application the treated area of skin became hyperemic, and this was followed soon after by a localized edematous swelling. These changes, evident after every application and persisting to some extent during the intervening periods, began to assume relatively less prominence as the epithelium became thickened and covered with loosely adhering, glistening flakes of keratin. This flaky hyperkeratosis became progressively more pronounced with each application.

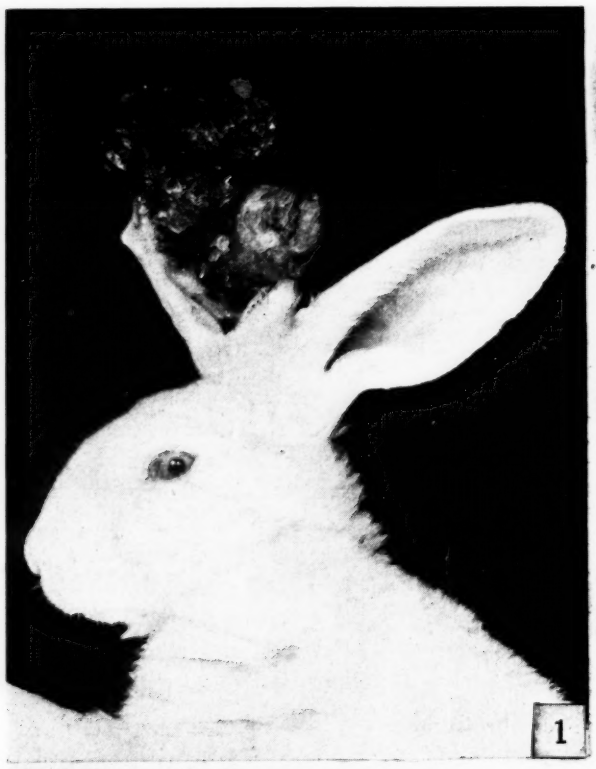
Warts began to arise at the site of application at an early date, the first tumors appearing after 5, 5½, 6, 7, and 9 weeks respectively in the 5 rabbits. Many more tumors subsequently developed in all cases, until the inner surface of each treated ear became filled with a crowded collection of excrescences of different shapes and sizes, and of varying degrees of cornification. Many of these became confluent, and in later stages acquired the character of massive horny outgrowths (Fig. 1). In 1 rabbit the growths remained benign till the end of the experiment (26 weeks), but unmistakable signs of malignancy became apparent in the other 4 after the 16th, 16th, 19th, and 21st weeks respectively.

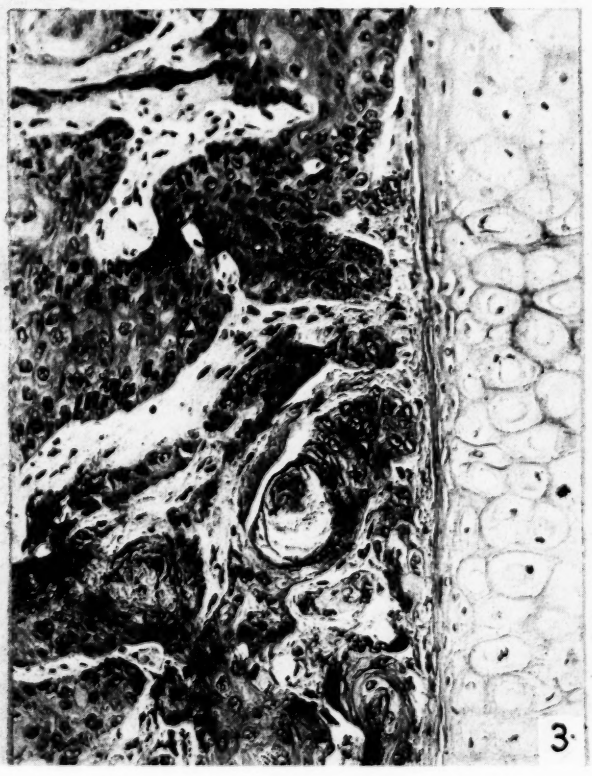
Subsequent histological examination of the lesions confirmed the malignant nature of the growths, as evidenced by their anaplastic character, deep penetration down to the cartilage (Figs. 2, 3, and 4), and in 2 cases through the cartilage to the other side. One of these led to the development of a large tumor on the outer side of the ear, which finally ulcerated to the surface. Metastases were not found in any of the animals.

Tar (diluted with a little benzene for ease of application) was applied for 36 weeks to the left ears of 6 rabbits. These rabbits also received applications of 25 per cent turpentine in acetone for the same period to the right ears. No tumors appeared on the right ears, treated with turpentine, but on the left ears, treated with tar, warts began to arise after 8, 11, 12,

* Because of the difficulties of international communication the author has not read proof of this article.

[1] See, however, a recent report (13) on the development of carcinomas in cottontail rabbits at the site of *injection* of 20-methylcholanthrene.





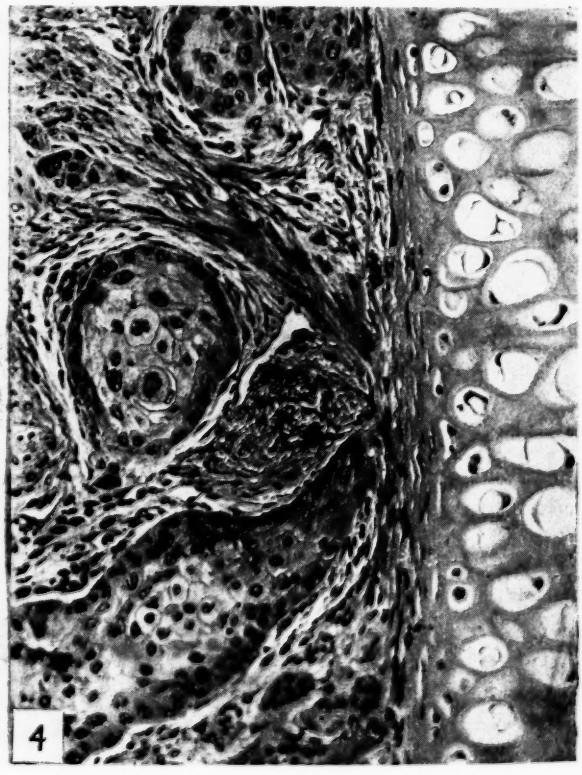


FIG. 1.—*Rabbit 71.* 9,10-Dimethyl-1,2-benzanthracene twice weekly to the right ear for 24 weeks. Note massive horny growth protruding from right ear, and evidence of its extension through the cartilage to outer side of ear.

FIG. 2.—*Rabbit 71.* Part of tumor shown in Fig. 1. Note highly keratinizing character of growth. Mag. × 25.

FIG. 3.—*Rabbit 71.* Same as Fig. 2; higher magnification. Note invasion of carcinomatous growth down to the cartilage. Mag. × 150.

FIG. 4.—*Rabbit 72.* 9,10-Dimethyl-1,2-benzanthracene twice weekly to right ear for 26 weeks. Another squamous cell carcinoma showing invasion down to the cartilage. Mag. × 150.

12, 16, and 29 weeks respectively. Neither the hyperemic nor the edematous changes were as intense as in the case of 9,10-dimethyl-1,2-benzanthracene, and the hyperkeratosis, though well developed, was less flaky in character. Multiple warts developed in the ensuing weeks, but they were few in number, their rate of growth was slow, and malignancy developed in only 1 case, at about the 30th week of application.

3,4-Benzpyrene (0.5 per cent in acetone, benzene, or xylene). This section includes an assorted collection of experiments in which benzpyrene was applied as part of an investigation unconnected with the present work. However, they may conveniently serve as controls for the present work, since the animals used were of the same stock and kept under similar conditions of diet, housing, and so forth.

First series.—In 6 rabbits the left ears were treated for 17 weeks with benzpyrene dissolved in benzene, and then for 16 weeks with the same substance dissolved in acetone. In 4 of these rabbits the right ears were treated for 47 weeks with benzpyrene in acetone, while the other 2 received an intravenous injection of 10 ml. of a saline extract of Shope papilloma virus.

None of these animals developed any tumors.

Second series.—In 7 rabbits the right ears were treated with benzpyrene in xylene for periods ranging from 39 to 48 weeks. At the same time the left ears were treated as follows: in 2 rabbits, applications of benzpyrene in acetone for 14 weeks with no further treatment; in 3 rabbits, the same treatment for 39 weeks; in the remaining 2 rabbits, the same treatment for 4 weeks, followed by applications of 5 per cent croton oil in acetone for a further 10 weeks.

A small wart developed in one of the rabbits after 33 weeks, at the site of application of benzpyrene in acetone; a wart appeared also in another rabbit after 48 weeks, at the site of application of benzpyrene in xylene. No other tumors were observed.

Thus among the 13 rabbits (20 ears receiving benzpyrene for 33 to 48 weeks) tumors were observed in only 2 cases.

DISCUSSION

The present investigation is concerned primarily with the carcinogenic action of 9,10-dimethyl-1,2-benzanthracene on the rabbit's skin, and the results obtained with 3,4-benzpyrene and with tar are presented merely for comparison. It would be desirable, therefore, to check the results for tar and benzpyrene with those of other workers.

Owing to variations in composition, and therefore in potency, of the different samples of tar used by various workers, published results on its carcinogenic action are very varied (14), and it is obviously impossible to quote a standard potency for this agent. Most observers would agree, however, that warts often appear early in rabbits after tar painting, but that such warts do not readily become malignant as they do, for instance, in mice. Hence when warts begin to arise from about the eighth week of application, as obtained in the present tar experiment, the tar in question may be considered as of more than average potency.

With regard to 3,4-benzpyrene, published results of carcinogenicity tests on rabbits are also conflicting, despite the fact that this substance is a single chemical compound. Thus benign and even malignant tumors have been produced by some workers (4, 5, 11, 12), yet not by others (6, 7, 9, 10). It is probable, however, that the failures were due to inadequate duration of the painting. The most reliable data seem to be those of Schürch (11), who painted 20 rabbits with benzpyrene until all the survivors had acquired tumors. He obtained benign growths in 6 to 14 months, with an average period of 11 months, and malignant tumors in 13 to 45 months, with an average period of 27 months. In the present experiments with benzpyrene, most of the animals were treated for less than 11 months, and the yield of tumors (2 among 20 ears of 13 rabbits) seems somewhat low by comparison, though probably not significantly so.

The results of the present investigation are of interest also from the point of view of species response. For purposes of comparison, the available data concerning the response of the skin of the mouse and rabbit respectively to the 3 carcinogens dealt with in the present paper are summarized in Table I. These results show: (a) that 3,4-benzpyrene is much less effective on the rabbit than on the mouse, as regards both benign and malignant tumor production; (b) that tar differs from benzpyrene in being equally effective for the two species as far as benign tumor production is concerned, though less effective for the rabbit as regards the induction of malignancy; and (c) that 9,10-dimethyl-1,2-benzanthracene differs from both tar and benzpyrene, since it is equally effective for the two species as regards both benign and malignant tumor production.

From this it would appear that the relative deficiencies in carcinogenic action of tar and of benzpyrene on the rabbit result from peculiarities of these substances, rather than from a general unresponsiveness to carcinogenesis on the part of the rabbit's skin.

SUMMARY

9,10-Dimethyl-1,2-benzanthracene is highly carcinogenic for the rabbit's skin, producing multiple, progressively growing warts after 5 weeks' application,

TABLE I: RELATIVE CARCINOGENIC POTENCY FOR THE SKIN

	Benign tumors		Malignant tumors	
	Mouse	Rabbit	Mouse	Rabbit
Tar	+++++	++++	+++	++
3,4-Benzpyrene	++++	++[a]	+++	±[a]
9-10-Dimethyl-1,2-benzanthracene	+++++[b]	+++++	+++++[b]	++++

+++++ = first tumor appearing in 5 weeks or less;
++++ = " " " " 6-15 weeks;
+++ = " " " " 16-25 " ;
++ = " " " " 26-35 " ;
+ = " " " " 36-45 " ;
± = " " " later than the 45th week.

The values marked (a) are based on the results of Schürch (11); those marked (b) on the results of Bachmann *et al.* (1); all other values on the author's own results.

and malignant tumors after about 16 weeks' application.

By comparison 3,4-benzpyrene is a very weak carcinogen for the rabbit's skin, while tar, though fairly potent in the sense of inducing early warts, is relatively weak when judged on the basis of continued growth and development of malignancy.

I am indebted to Mr. F. L. Warren, of the Chester Beatty Research Institute, the Royal Cancer Hospital, London, for a generous gift of 9,10-dimethyl-1,2-benzanthracene, and to the Yorkshire Tar Distillers, Ltd. for the horizontal retort tar used in these investigations. The 3,4-benzpyrene was supplied by Hoffmann-La Roche & Co., Ltd. My thanks are due to Mr. W. H. Wheal for valuable technical assistance and the care of the animals, and to Mr. H. Axtell for the photographic work.

ADDENDUM

An experiment still in progress further illustrates the high carcinogenic potency of 9,10-dimethyl-1,2-benzanthracene for the rabbit's skin.

Ten rabbits were treated with a 0.5 per cent solution of this compound, application being made only once a week to the skin of the back after removal of the hair each time with electric clippers.

The first papilloma appeared after 5 weeks. By the end of the eighth week tumors were already present in 6 of the 10 rabbits. In 4 of these the papillomas were single; in the other 2, they were multiple.

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The Heterologous Transplantation of Mouse and Rat Tumors*

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Several years ago it was shown that human and rabbit cancers can be readily transferred to animals of alien species by utilizing the anterior chamber of the eye as a transplantation site (2-4). The study of heterotransplantability has since been extended in various directions, and one phase of the investigation has been an examination of cancers of other species with respect to this property. Cancers from a variety of species have been successfully transplanted to guinea pigs and rabbits, but tumors of mice and rats formed the bulk of available material and have been studied from several viewpoints in relation to their ability to survive and grow in foreign hosts. The present paper will report the results of heterologous transfer of a number of propagable growths of rats and mice used in cancer research, while later papers will be concerned with a study of the developmental course of spontaneous and experimental mouse tumors with especial reference to the evolution of autonomy as expressed by heterotransplantability.

The extensive literature dealing with the heterologous transplantation of mouse and rat tumors was reviewed in 1933 (8). Successful transfer between species was undoubtedly effected in several instances. Thus Murphy transferred the Jensen rat sarcoma to the developing chick embryo and was able to carry the tumor serially in this host (9); transfer back to the rat resulted in takes, but all attempts to transplant the tumor to adult chickens failed. Shirai reported briefly the transplantation of a rat sarcoma to the brains of adult mice, but apparently serial transfer was not attempted (11). Putnoky transplanted Ehrlich's mouse carcinoma subcutaneously into adult rats and has maintained the tumor by serial transfer since 1929 (10). A large inoculum of 300 to 500 mgm. of tumor is used, and growth is rapid. Regression begins by the tenth day, and transfer must be made at this time. Histologically the appearance of the tumor is identical with that in the mouse host.

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** Fellow of The Jane Coffin Childs Memorial Fund for Medical Research.

MATERIALS AND METHODS

The mouse tumors [1] used in the present experiments consisted of an epidermoid lung carcinoma, sarcoma 180, an ovarian embryoma, an experimentally induced hepatoma, and 5 different mammary carcinomas. The rat tumors were R39 and 2426.

The anterior chamber of the eye was used as a transplantation site and the technic employed has been described in detail (5). Testicular and intramuscular transfers were performed in several instances, and both tissue fragments and cellular emulsions were employed.

Guinea pigs, rabbits, rats, hens, and ducks served as foreign hosts. Animals of both sexes and of different age groups were used, with no significant variation in the results obtained. The animals were not subjected to special treatment before or after transfer but were maintained under ordinary conditions of cage life.

Before heterologous transfer, all the mouse tumors were tested to determine their transplantability in various mouse breeds. The breeds used were the C3H, C57 black, dba, and the A and Bagg albinos.

RESULTS

The results of transfer of the different mouse and rat tumors to the anterior chambers of the eyes of guinea pigs are summarized in Table I. The tumors varied in growth rate and in behavior in the foreign hosts, and will be described separately.

Bronchogenic Carcinoma

This tumor, which arose spontaneously in a bronchus of a CBA mouse and was characterized histologically by prickle cells, has been carried by serial subcutaneous transfer in this strain by Dr. Gardner. It grows equally well in C3H mice, and has been maintained in this line in our laboratory. Takes occur regularly

[1] These tumors were made available through the courtesy of Dr. William H. Woglom, Department of Cancer Research, Columbia University; Dr. William U. Gardner, Department of Anatomy, Yale University School of Medicine, and Dr. Austin M. Brues, Massachusetts General Hospital, Boston, Massachusetts.

in 100 per cent of the animals used, and the transplants grow rapidly. It is invasive and extends through the abdominal muscles and lymphatics to involve the peritoneum in 3 to 4 weeks. Metastasis to the lungs and other viscera is common.

On test, takes were obtained by subcutaneous transfer in dba's, A's, and Bagg albinos, but not in C57 blacks. There were relatively few takes in the first generation transfers to the new strains, but with serial passage the transplantability increased and after several generations equalled that obtained in the parent line. No significant difference in the morphology or behavior of the tumor was noted in the new strains.

growth is progressive; the cornea ruptures, and the tumor protrudes externally as a fungating mass.

The factors concerned in regression have been studied in a series of reinoculation experiments in which living tumor was transferred to the anterior chamber of the opposite side after regression of the first transplant. If the transplant regresses before filling the chamber, the animal is invariably found resistant to further transfer. A similar situation occasionally obtains when regression follows the complete filling of the chamber, but in the majority of such cases transfer to the second eye results in takes. It is suggested, therefore, that in the former instances

TABLE I: THE RESULTS* OF TRANSFER OF MOUSE AND RAT TUMORS TO THE ANTERIOR CHAMBERS OF THE EYES OF GUINEA PIGS

Generation number	Mouse tumors												Rat sarcoma 39	
	Bronchogenic carcinoma		180		Embryoma		Hepatoma		RC		Yale			
	No. of pigs	No. of takes	No. of pigs	No. of takes	No. of pigs	No. of takes	No. of pigs	No. of takes	No. of pigs	No. of takes	No. of pigs	No. of takes	No. of pigs	No. of takes
1	6	3	13	7	21	15	15	3	16	9	24	12	35	16
2	6	1	8	5			7	2	4	2	9	5	8	4
3	4	1	12	8			6	5	6	5	7	5	4	3
4	6	1	19	17			6	6	5	5				
5	6	6	11	9			6	6	6	6				
6	15	10	16	13										
7	14	5	7	3										
8	8	5	5	5										
9	11	10	5	5										
10	12	6												
11	8	5												
12	12	10												
13	8	6												
14	6	5												
15	4	4												
16	5	5												
17	5	5												
18	6	6												
	142	94	96	72	21	15	40	22	37	27	40	22	47	23

* Tumors 755, BR, ST, and 2426 did not take in 33, 30, 30, and 25 guinea pigs respectively.

Transfer to the anterior chamber of guinea pig eyes.—The tumor has now been carried by serial anterior chamber passage in guinea pigs for more than a year. Growth occurred in approximately 66 per cent of the animals in the entire series, but an examination of succeeding generations shows a gradual increase in the incidence of takes, to reach 100 per cent in the last 4 passages.

Growth and vascularization of the transplants are invariably evident by the tenth day following transfer, and occasionally the tumor increases to fill one-half of the chamber in this period. In rare instances regression follows a short period of growth, but in the great majority of cases the transplant enlarges to fill the chamber by the 20th day. Thereafter partial regression followed by renewed growth or complete and permanent regression may occur. In other animals regression results from the development of an immune state, whereas in the second case its occurrence is related to the diminished blood supply incident to rapidly expanding growth in a confined space. The histological appearance of regressing transplants offers contributory evidence, for necrosis is widespread in the first type whereas in the second type surviving cells are present in a perivascular position. There is no indication that regression bears any relationship to the alien nature of the transplant, for identical phenomena are observed in homologous transfers.

Histologically the transplants in mice and guinea pigs are similar in appearance (Figs. 1 and 2). Occasionally in mice the character and arrangement of the tumor cells are suggestive of sarcoma, but the true epithelial nature of the growth becomes evident on examination of guinea pig transplants. It appears to

be a general rule that growth in the guinea pig is associated with a slightly higher degree of differentiation and organization than is observed in the primary host. Guinea pig transfer is thus of considerable value in the classification of anaplastic, poorly organized tumors, and is frequently used in our laboratory as an aid to the microscopic diagnosis of human tumors.

The tumor cells invade the guinea pig's iris early in the course of growth, and may extend deeply into its substance before an increase in the circumference of the transplant is noted. This is apparently not associated with invasion of vascular walls, for hemorrhage is much less common than in the case of rat sarcoma 39, which also is characterized by early iris invasion.

Growth in the bulk of the transplant is accompanied by an extension of tumor cells over the surface of the iris in the manner of a lining membrane. Invasion occurs in many areas but does not extend into its retinal portion. Subsequent growth is purely expansive, and the bulging posterior surface of the iris is invariably covered by stretched but intact retina.

After rupture of the cornea the tumor extends under the bulbar conjunctiva and invades the eyelid. Infection invariably follows and necessitates sacrifice of the animal.

Transfer to guinea pig testicles.—Testicular transfer of the tumor is readily accomplished, either with tissue obtained directly from the mouse or after growth in the guinea pig's eye. The growth rate of the transplants varies widely, and occasionally exceeds that observed in the anterior chamber. Usually the testicular parenchyma is completely replaced by the 40th day, but in some instances the growth persists as a pea-sized nodule for many months.

Histologically the testicular and anterior-chamber transplants are identical in cellular character and structure (Fig. 3). The tumors are sometimes surrounded by a connective tissue capsule containing numerous eosinophils, and growth appears to be expansive in nature. In other animals, however, the tumor has no discrete boundary, but extends in strands and columns throughout the parenchyma, invading and destroying the tubules (Fig. 4).

Transfer to the anterior chamber of rabbit eyes.—Takes in rabbits' eyes have been obtained with mouse tumor tissue as well as with fragments of guinea pig transplants, but the latter material has proved most satisfactory. Curiously, a higher percentage of takes results if the mouse tissue used for transfer is obtained from a first generation tumor in an alien strain, such as the A albino, rather than from the C3H strain in which the stock tumor is maintained by serial passage. In any case, regardless of derivation, the rabbit's eye is much less favorable than the guinea pig's as a site for growth of the tumor, and takes occur in less than a quarter of the animals used.

The sequence of events after vascularization is similar to that described in the guinea pig, and the transplants show no significant change in morphology (Figs. 5 and 6).

Transfer to rabbit testicles.—Transfer to rabbits' testicles has been attempted only with tissue derived from guinea pig anterior-chamber transplants. Takes occur in approximately 25 per cent of the animals used, and the behavior of the tumor is comparable to that observed in the guinea pig's testicle. Histologically the growths often tend to resemble the more sarcomatous appearing areas occasionally noted in mouse tumors (Fig. 7).

Transfer to the anterior chamber of rat eyes.—Tumor tissue has been transferred directly from mice to rats' eyes with a high incidence of takes. Growth is very rapid, and the chamber may be filled with tumor by the eighth day. However, the transplant is invariably infected, and abscess formation has been the ultimate fate of the tissue in all the animals held for more than a month.

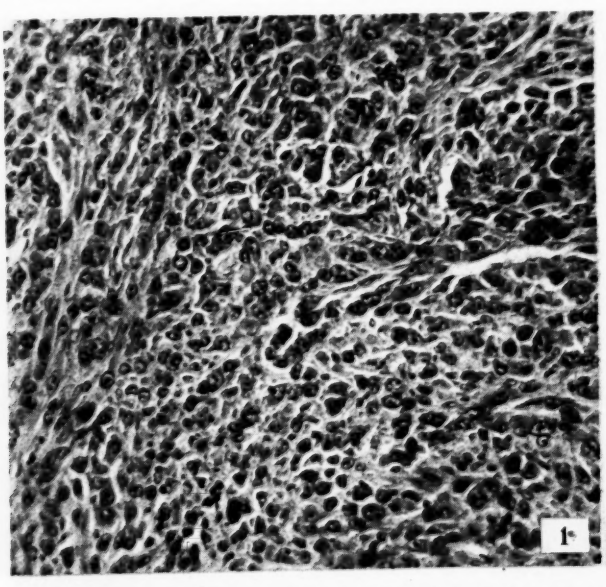
The transplants in animals killed early in the course of growth show large areas of necrosis containing scattered islands of living tumor cells. The cells and their nuclei are more variable in size and shape than in other species, and multinucleate forms are common. Generally the cells tend to be large and their arrangement is distinctly epithelial (Fig. 8).

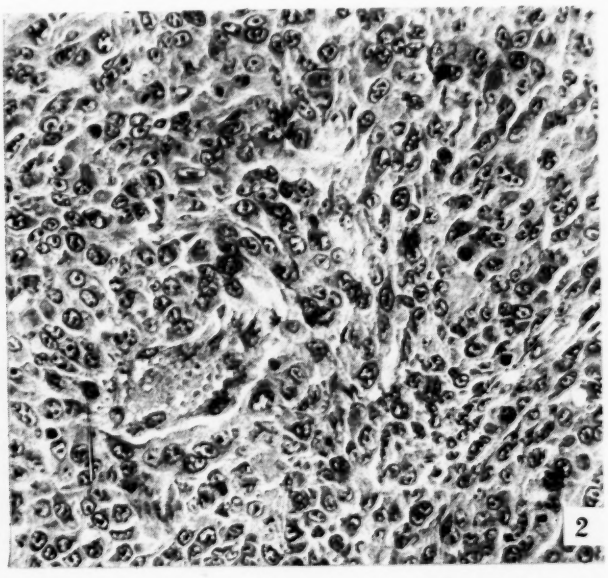
Transfer to the anterior chamber of hen and duck eyes.—Surprisingly, transfer of the tumor to the eyes of hens and ducks gives rise to nearly 100 per cent of takes, and identical results are obtained regardless of the derivation of the tissue. Vascularization of the fragments is apparent within 2 weeks of transfer, but subsequent growth is extremely slow and the transplants may not increase by more than 3 diameters in the following 4 months.

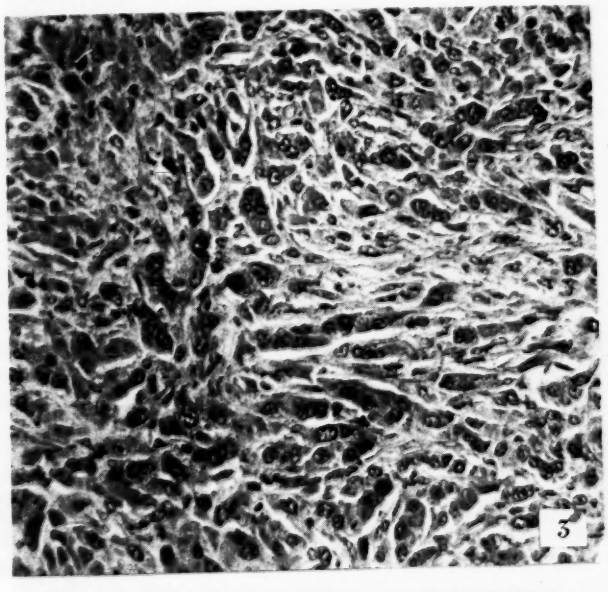
The transplants have been studied histologically at different periods of growth. Between the first and second weeks the great majority of tumor cells die and a large portion of the transplant is converted into a necrotic, amorphous mass. However, islands of cells in scattered areas survive and grow (Fig. 9). The surviving cells are large, contain abnormal nuclear forms, and mitosis is common. At a later period the graft is made up entirely of living cells with supporting stroma and blood vessels. The cells are smaller than usual, largely because of a diminution in the amount of cytoplasm, and are closely packed (Fig. 10).

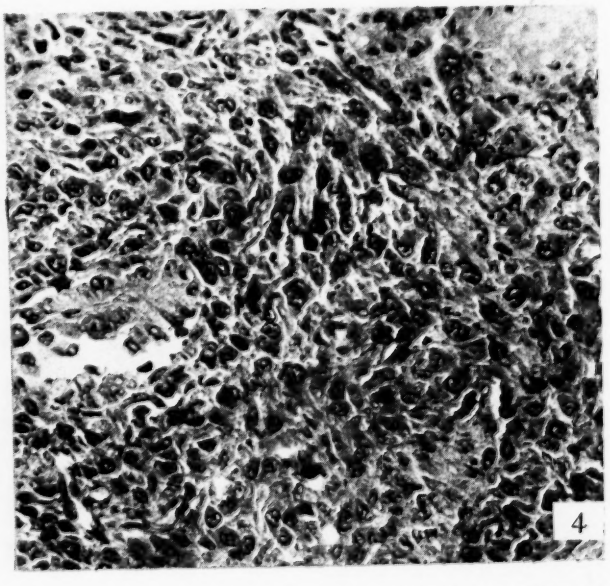
Tumor 180

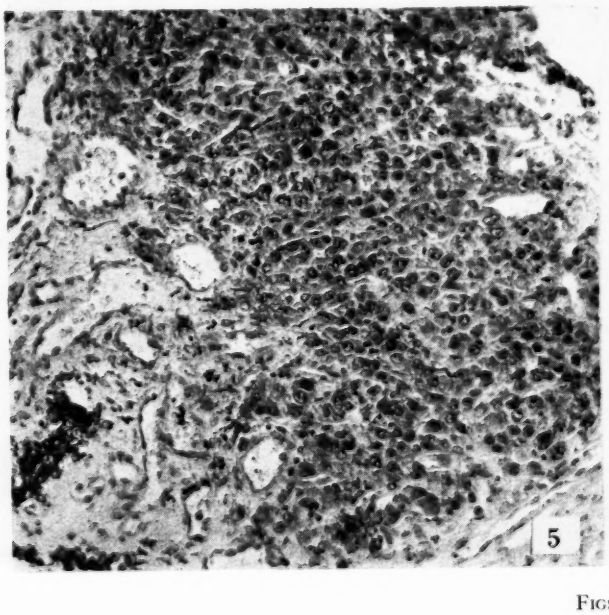
Mouse tumor 180, propagated for many years in the Department of Cancer Research of Columbia Uni-

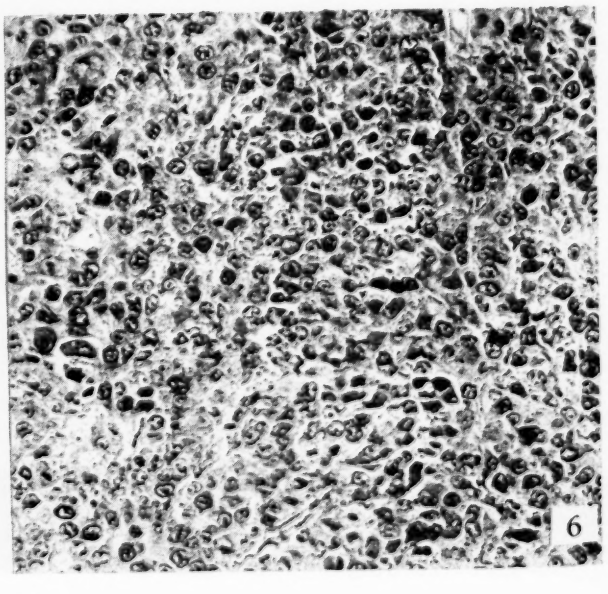












FIGS. 1-6

versity, is widely used in experimental work. It is undoubtedly a sarcoma, and there is a possibility that it is actually the old Ehrlich sarcoma. It has been grown in many different strains of mice, and in our laboratory proved readily transplantable to dba's, C57's, A's, and Bagg albinos. For stock purposes the tumor is carried in C3H mice, where it grows regularly in 100 per cent of the animals. In this strain it grows rapidly and is invasive. The abdominal wall is invaded and peritoneal extension occurs in from 3 to 4 weeks after subcutaneous transfer. The growths frequently ulcerate, with consequent infection, and death usually occurs before metastasis. However, metastasis eventually takes place in animals that survive infection or peritoneal extension.

Transfer to the anterior chamber of guinea pig eyes.—Transfer of the tumor to the eyes of guinea pigs gives rise to growth in 75 per cent of the animals used but, as in the case of the lung carcinoma, the frequency of takes increases with continued passage.

The transplants undergo considerable increase in size before vascularization becomes evident, and subsequent growth is so rapid that the chamber may be completely filled within 8 days of transfer. Thereafter, regression invariably occurs. Regression may be rapid and result in the death of the transplant in 24 hours but is sometimes delayed so that foci of living, transplantable cells persist for 3 or 4 weeks. Neither corneal rupture nor renewed growth has been observed.

Histologically the transplants are indistinguishable from the original mouse tumor (Figs. 11 and 12). Sections of early grafts are devoid of blood vessels, suggesting that primary growth may occur in the manner of a tissue culture, the tumor cells imbibing nutriment directly from the surrounding aqueous humor. At a later stage, however, the growths are abundantly supplied with thin-walled blood vessels. Invasion of the iris is a constant feature, but does not extend beyond the pars iridica retinae.

Transfer to guinea pig testicles.—Testicular takes occur after transfer of tumor derived from the mouse or the guinea pigs' anterior chamber, but in the great majority of cases the transplants regress within 10 days. In 3 of 25 cases the growths persisted and increased to the size of marbles in a period of 30 days. One of these animals was killed for microscopic study, while the remaining 2 were held to determine the eventual fate of the tumor. In both instances the tumors decreased in size and no trace remained on palpation of the testicles on the 50th day.

Histologically the essential tumor cells in early transplants are identical in structure and arrangement with those found in the eye, but in contrast to the situation in the anterior chamber the foci of growth are surrounded and compressed by capsules of dense fibrous connective tissue. A definite capsule was not observed in the 1 animal with persistent growth. Here tumor cells, many of which were in mitosis, were diffusely intermingled with young fibroblasts and strands of older connective tissue. The mass composed of these elements was not circumscribed but of irregular outline, with projections extending into adjacent testicular parenchyma, engulfing and destroying tubules in a manner comparable to that observed in the case of the previous tumor. Remnants of tubules were also present in the center of the tumor, suggesting that in this instance growth had been infiltrative rather than purely expansive as in the earlier transplants.

Intramuscular transfer in guinea pigs.—Growth has followed transfer of the tumor to the thigh muscles in 8 of 15 animals. The transplants increase in size with extreme rapidity and by the tenth day the thigh is converted into a diffuse incapacitating mass extending from hip to knee joint. However, regression occurs with the same rapidity and no clinical evidence of tumor remains on the 25th day.

Histologically the appearance of the transplants is comparable with that of persistent testicular growths. In scattered areas the tumor cells are aggregated in masses, but elsewhere are diffusely interspered with young fibroblasts and muscular elements (Fig. 13).

Transfer to the anterior chamber of rabbit eyes.—Transfer to rabbit eyes has been successful in only about 10 per cent of cases (3 of 24). The clinical course of the tumor is comparable with that observed in guinea pigs, and its histological appearance is identical (Fig. 14).

Transfer to the rabbit's testicle.—Transfer to the

DESCRIPTION OF FIGS. 1 TO 6

All sections stained with hematoxylin and eosin.

FIG. 1.—Transplant of bronchogenic carcinoma growing in subcutaneous tissues of C3H mouse. Mag. × 275.

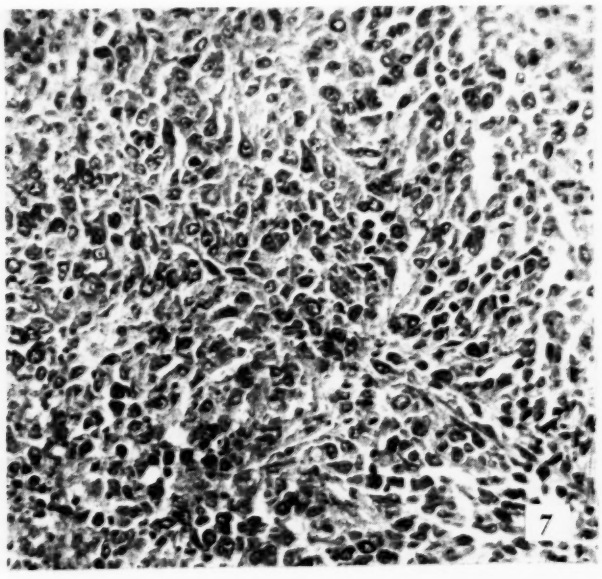
FIG. 2.—Transplant of bronchogenic carcinoma growing in anterior chamber of guinea pig's eye; 15th serial passage. Note thin-walled blood vessel in lower midportion of photograph. Mag. × 275.

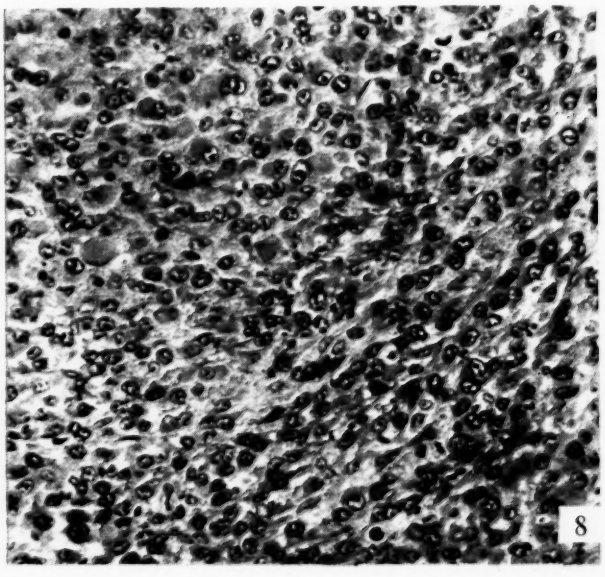
FIG. 3.—Transplant of bronchogenic carcinoma growing in testicle of guinea pig. Mag. × 275.

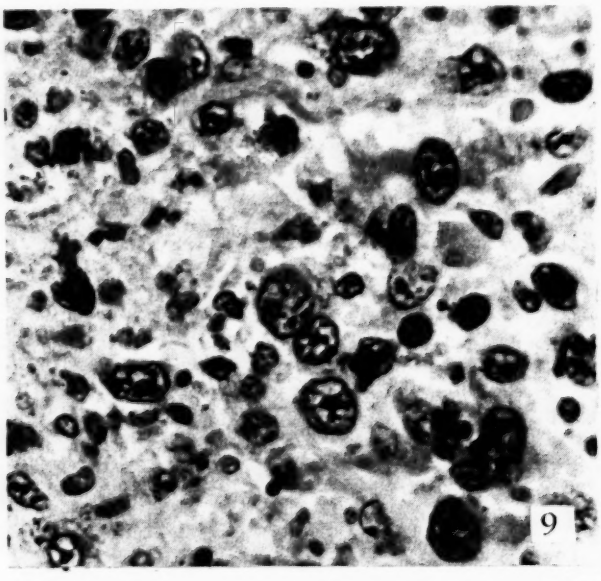
FIG. 4.—Transplant of bronchogenic carcinoma growing in testicle of guinea pig. Note destruction of tubules in upper right and midleft portions of photograph. Mag. × 275.

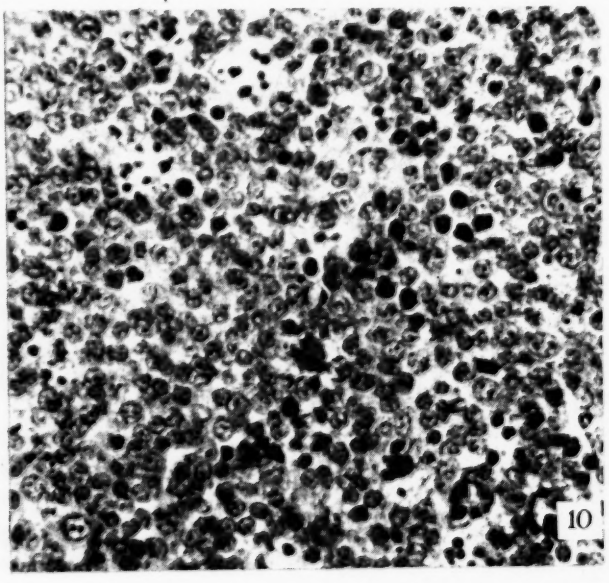
FIG. 5.—Transplant of bronchogenic carcinoma growing in anterior chamber of rabbit's eye. Note invasion of iris. Mag. × 200.

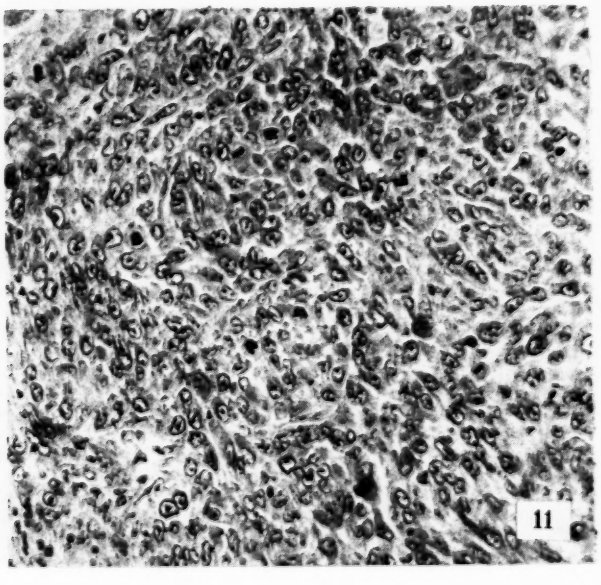
FIG. 6.—Transplant of bronchogenic carcinoma growing in anterior chamber of rabbit's eye. Mag. × 275.

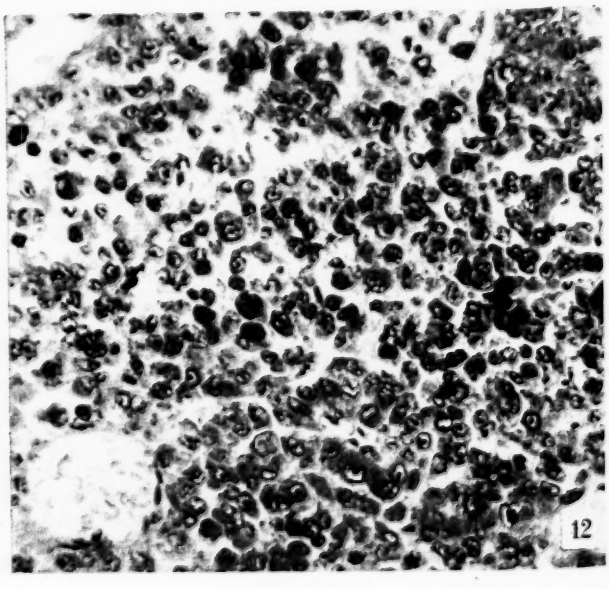












FIGS. 7-12

testicle with tissue derived from both rabbit and guinea pig eyes has been uniformly unsuccessful, no takes having been obtained in more than 30 attempts.

Transfer to the anterior chamber of rat eyes.—Anterior-chamber transfer results in 100 per cent of takes in rats, but, as in the case of the lung carcinoma, the transplants are invariably infected and undergo a similar course (Fig. 15).

Ovarian Embryoma

This tumor arose in the ovary of a C3H mouse and has been carried serially in this strain by Jackson and Brues. Takes occur on subcutaneous transfer in approximately 75 per cent of animals but the growth rate varies widely. The tumor is composed of a mixture of embryonal and mature tissues, which persist on transfer with variation of the predominant cell type in different animals (7).

The tumor has been carried in C3H mice in this laboratory and has also been successfully transplanted to the A albino strain. Its histological characteristics in our C3H mice are identical with those described by Jackson and Brues, but in A albinos the growth consists of sheets of embryonal cells without architectural organization (Figs. 16 and 17). Growth of the tumor is largely expansive, with minimal muscular invasion. Lymphatic extension has not been observed, but metastasis to the lungs and other viscera occurs in the terminal stages.

Transfer to the anterior chamber of guinea pig eyes.—Experiments involving the heterologous transplantation of this tumor have been limited to the guinea pig's eye, and the tumor has not been carried serially in this species. First generation transfers from 4 separate mouse tumors have given rise to growth in 15 of the 21 animals used, or approximately 70 per cent.

Growth of the transplants is generally much more rapid than in the natural host, and in many instances the anterior chambers of guinea pigs' eyes are completely filled with tumor by the 11th day, at least a week before growth can be detected in control mice. On the other hand, growth may be greatly retarded; in one particular instance the transplant in an animal released to the breeding population as negative remained dormant for 3 months and then grew slowly to fill the chamber. In such cases the transplant may reach a large size and result in bulging of the cornea with destruction of the internal structures of the eye. Usually, however, after regression of the tumor the iris is little damaged and shows only a minor degree of scarring. Regression has been the eventual termination of the tumor in all animals held for continued observation.

In contrast to the parent tumors in C3H mice, the guinea pig transplants are composed of a single cell type. This is an embryonal cell suggestive of early embryonic epithelium, and appears to be identical with the elements producing the tumor in A strain mice. In the guinea pig, however, the cells are arranged in a definite architectural pattern closely imitating glandular acini (Fig. 18).

Hepatoma

This tumor is a liver carcinoma produced by *o*-amidoazotoluene in the Department of Medicine at Columbia University and carried serially by subcutaneous transfer in Bagg albino mice. In our laboratory it has also been transferred successfully to dba's, where it grows in approximately 50 per cent of the animals inoculated. Its morphology is identical in both mouse strains (Fig. 19). Growth is predominantly expansive, but muscular invasion occurs in late stages. Neither lymphatic extension nor metastasis has been observed.

Transfer to the anterior chamber of guinea pig eyes.—Study of this tumor in alien species has also been limited to the guinea pig's eye. Early transfers to this site gave rise to few takes, but the incidence of growth increased to 100 per cent in the fourth and fifth serial generations.

Vascularization of the transplants becomes evident within a week of transfer, but subsequent increase in size is slow and the chamber is rarely filled in less than 2 months. In occasional cases growth is further delayed, and may persist for as long as 150 days before regressive changes become noticeable. Early growth is almost entirely expansive in character and invasion of the iris

DESCRIPTION OF FIGS. 7 TO 12

Fig. 7.—Transplant of bronchogenic carcinoma growing in rabbit's testicle. Mag. × 275.

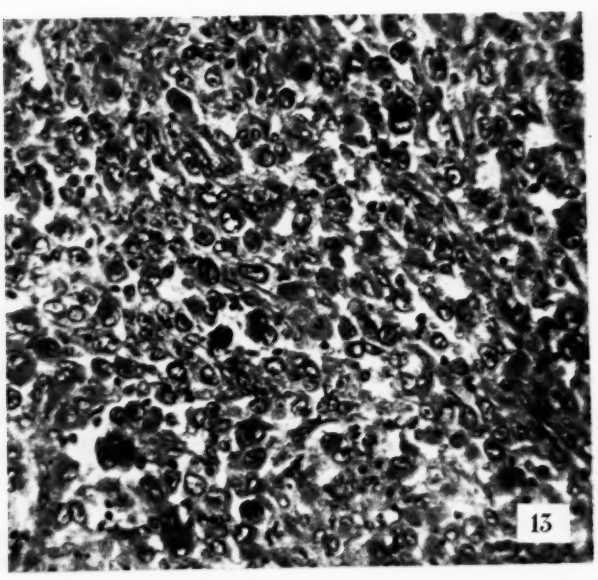
Fig. 8.—Transplant of bronchogenic carcinoma growing in anterior chamber of rat's eye. Mag. × 275.

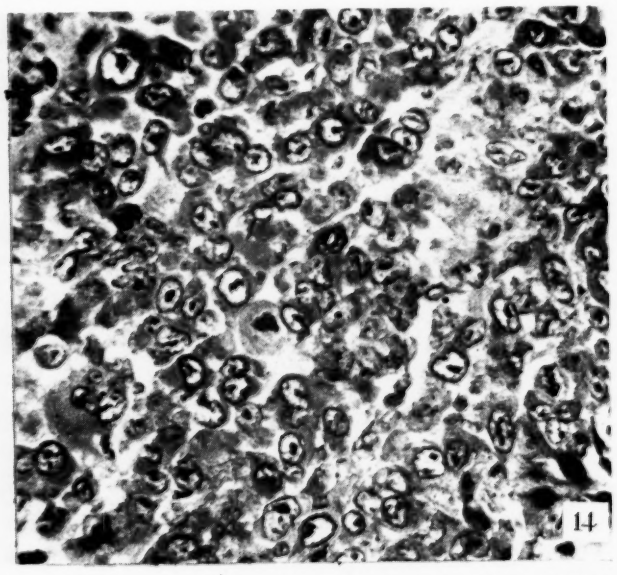
Fig. 9.—Transplant of bronchogenic carcinoma growing in anterior chamber of hen's eye, 2 weeks after transfer. Mag. × 650.

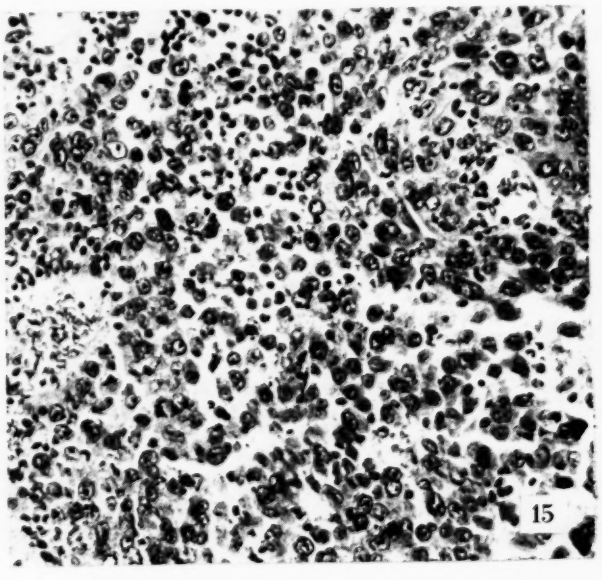
Fig. 10.—Transplant of bronchogenic carcinoma growing in anterior chamber of duck's eye, 3 months after transfer. Mag. × 340.

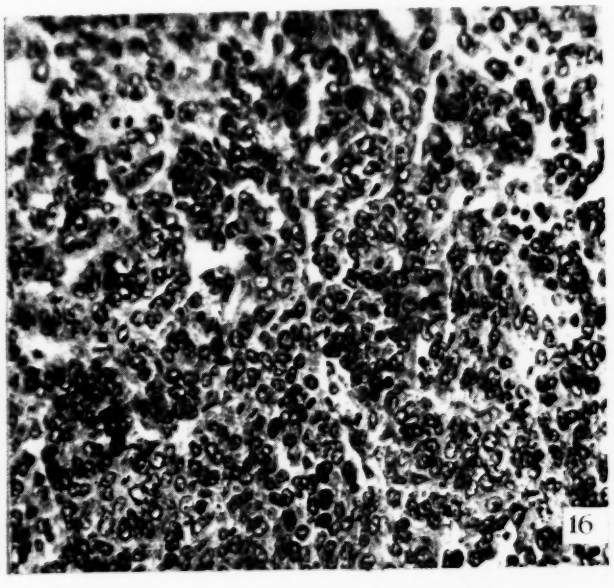
Fig. 11.—Transplant of sarcoma 180 growing in subcutaneous tissues of C3H mouse. Mag. × 275.

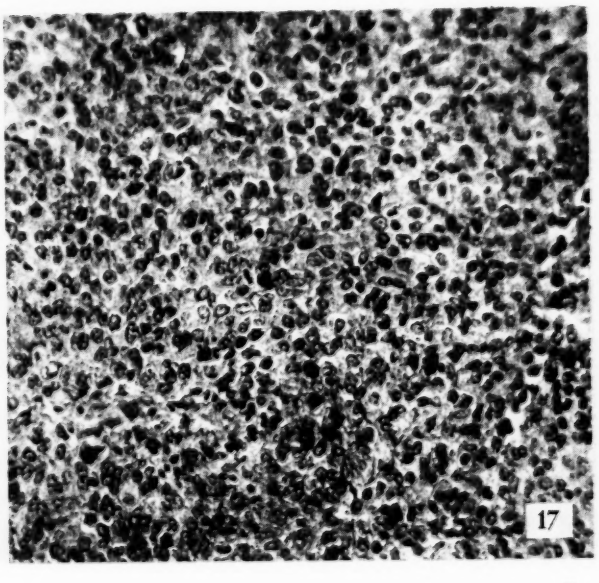
Fig. 12.—Transplant of sarcoma 180 growing in anterior chamber of a guinea pig's eye. Note vascularization. Mag. × 275.

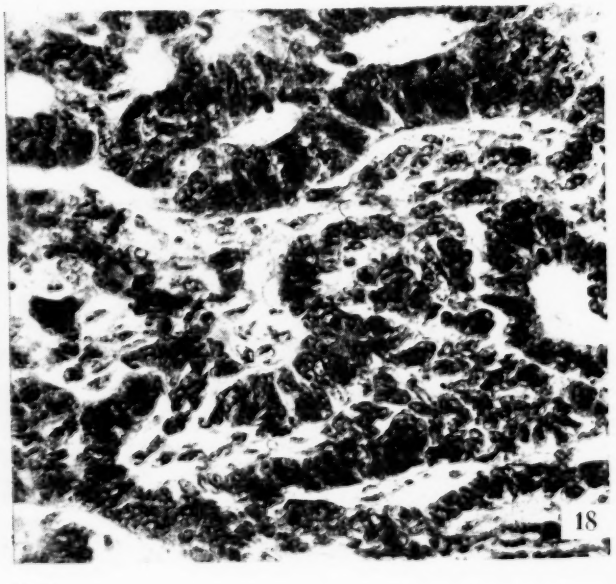












FIGS. 13-18

is never so pronounced as in the case of other tumors. After filling of the chamber regression is the rule, but renewed growth may occur in scattered foci at any time during the process. Histologically the transplants are identical with the mouse tumors in appearance (Fig. 20).

Mammary Carcinoma RC

This growth has been carried serially by subcutaneous transfer in dba mice at the Biochemical Institute of The University of Texas and in the Department of Cancer Research of Columbia University. In our laboratory it has also been transferred successfully to the C57 and A strains. The tumor grows expansively to produce a large subcutaneous mass with little muscular invasion. Invasion of the overlying skin, however, is a relatively constant feature, leading to ulceration with subsequent infection, and in the majority of cases death occurs without metastasis. Histologically the tumor consists of anaplastic epithelial cells arranged in solid rounded masses separated from each other by varying amounts of connective tissue stroma (Fig. 21).

Transfer to the anterior chamber of guinea pig eyes.—The tumor grows readily in the guinea pig's eye and 100 per cent of takes was obtained in the fourth serial generation. Growth is rapid and the chamber is almost invariably filled with tumor by the eighth day after transfer. Regression generally follows, as is usual with rapidly growing tumors, and is often succeeded by a period of renewed growth. In some cases the cornea ruptures at the site of the incision through which the graft was introduced, and the protruding tumor may survive for a number of weeks before infection and necrosis supervene.

The usual histological picture closely duplicates that found in the mouse (Fig. 22). Occasionally, however, there is an overgrowth of guinea pig connective tissue and the essential cancer cells are scattered singly or in small groups throughout the loose stroma, actually occupying only a relatively small part of the tumor mass.

Transfer to the anterior chamber of rat eyes.—Takes occur regularly in the anterior chambers of rats' eyes, but in our experience transfer to this species is always associated with infection of the transplants and the final picture is one of acute infection and abscess formation. Areas of growing tumor are found in animals killed during the first 2 weeks following transfer, and show the same morphological features observed in mice (Fig. 23).

Yale Tumor Number 1

This tumor is also a mammary carcinoma. It arose in an A strain mouse and has been under transfer at Yale for many years. It has been successfully transplanted also to mice of the C3H strain in our laboratory. The dominant mode of growth is expansive, but the tumor also invades and metastasizes. Histologically it is a well differentiated adenocarcinoma (Fig. 24).

Transfer to the anterior chamber of guinea pig eyes. Anterior-chamber transfer results in takes in approximately 50 per cent of the guinea pigs used, and despite its comparatively well organized structure the tumor grows with surprising rapidity. Vascularization and increase in size can usually be detected by the fourth day, and the chamber may be completely filled by the tenth. The growing tumor, like other well differentiated growths of glandular type, is characterized clinically by a cherry red color, which apparently reflects an abundant and complex blood supply. Regression and necrosis are easily detected by the appearance of white opacities, and their occurrence in scattered focal arrangement is a distinctive feature of the tumor during the third or fourth week of growth. These areas gradually coalesce and eventually involve all but a minute portion of the transplant. The opaque material slowly disappears over a period of weeks and the persistent pinkish tissue undergoes renewed growth.

Histologically the anterior chamber transplants are identical with the subcutaneous growths in mice (Fig. 25).

Mammary Tumor 755

This arose in the mamma of a C57 black mouse and has been maintained in this strain by serial subcutaneous transfer. Transplants grow slowly to produce a

DESCRIPTION OF FIGS. 13 TO 18

Fig. 13.—Transplant of sarcoma 180 growing in guinea pig's muscle. Mag. × 275.

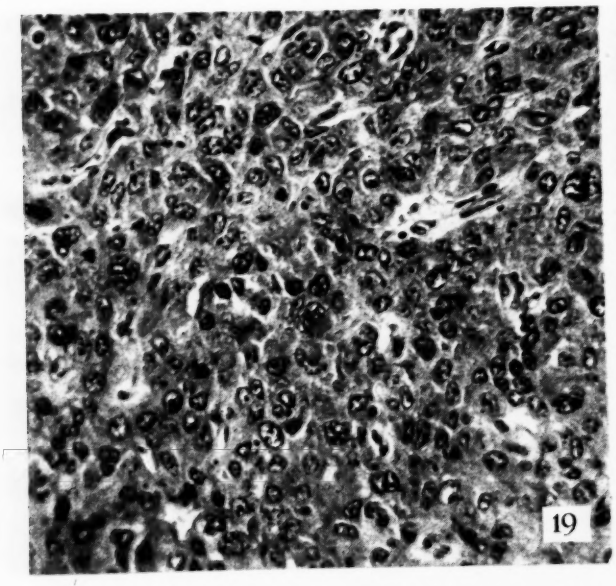
Fig. 14.—Transplant of sarcoma 180 growing in anterior chamber of rabbit's eye. Mag. × 340.

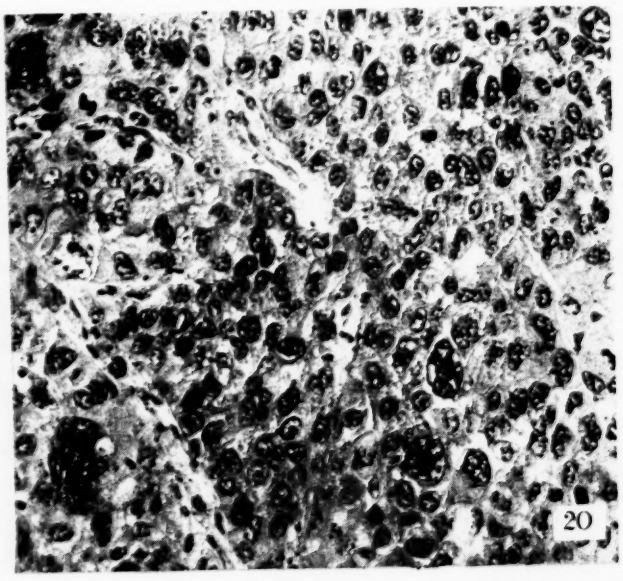
Fig. 15.—Transplant of sarcoma 180 growing in anterior chamber of rat's eye. Mag. × 275.

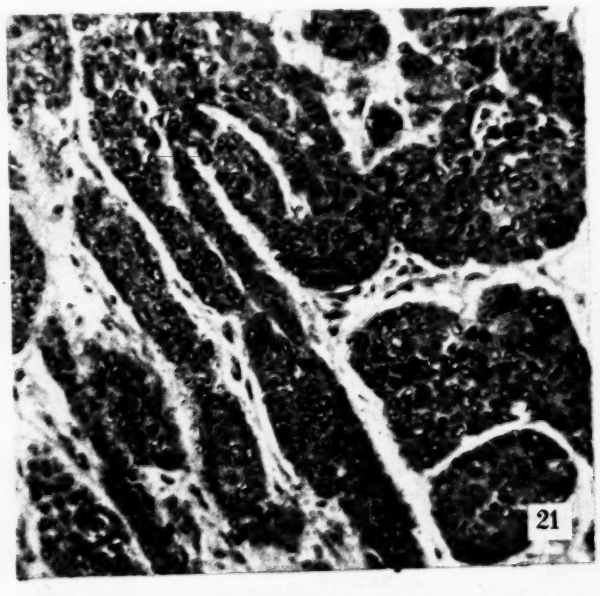
Fig. 16.—Transplant of embryoma growing in subcutaneous tissues of C3H mouse. The photograph represents the least organized area found in the growth, but even here a definite glandular pattern can be recognized. Mag. × 275.

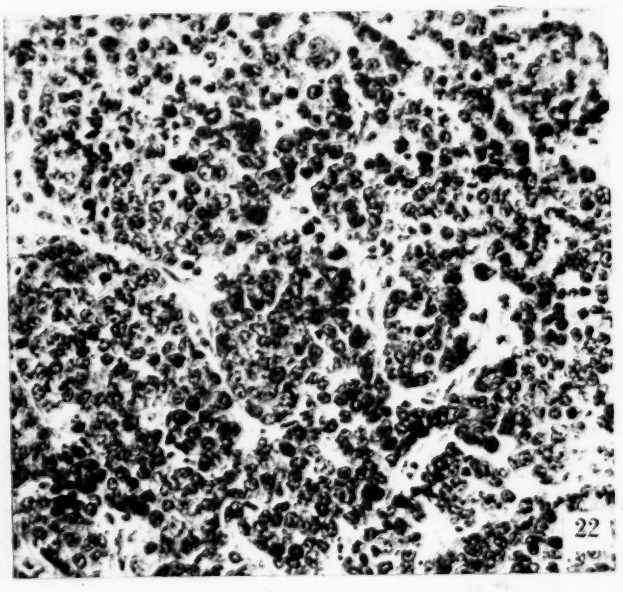
Fig. 17.—Transplant of embryoma growing in subcutaneous tissues of an A mouse. Note complete disorganization. Mag. × 275.

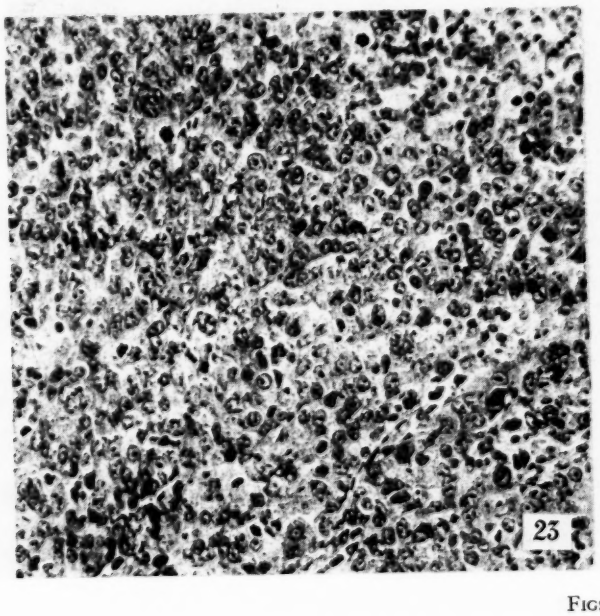
Fig. 18.—Transplant of embryoma growing in anterior chamber of guinea pig's eye. Appearance is that of a well differentiated adenocarcinoma. Mag. × 275.

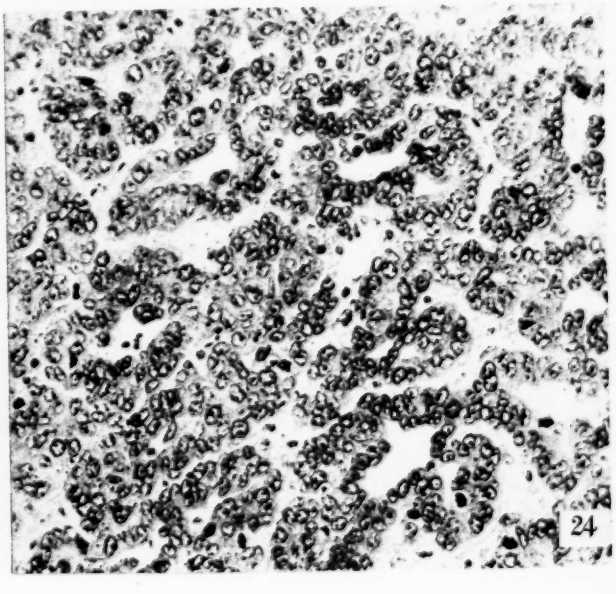












FIGS. 19-24

bulky tumor, but growth appears to be entirely expansive and neither invasion nor metastasis has been observed. Histologically there is only a slight suggestion of glandular pattern, and the essential cells are arranged in solid masses separated from one another by connective tissue septa.

In attempts to transfer the tumor to other lines of mice more than 50 animals of each strain, including the C3H, dba, A, and Bagg albino have been used, but in no instance has any suggestion of growth been noted. In like manner all attempts to transfer to guinea pigs and rabbits have been unsuccessful.

Mammary Tumor BR

This tumor arose in the mamma of a C3H mouse in our laboratory. A portion of the growth was removed at biopsy 2½ weeks after discovery, and has since been carried by serial subcutaneous transfer in the same strain. All attempts at transfer to other mouse strains, including C57, dba, A, and Bagg albino have been unsuccessful. In C3H mice growth is entirely expansive, and neither lymphatic extension nor metastasis has been observed. Histologically the tumor is a well differentiated adenocarcinoma.

Attempts have been made to transfer this tumor from 3 different mice bearing it to the anterior chambers of guinea pigs' eyes. Ten pigs were used in each transfer, and all were held under observation for a period of 2 months. No indication of growth was observed in any instance.

Mammary Tumor ST

This tumor arose in the mamma of a C3H mouse and has been carried by serial transfer in Dr. Gardner's laboratory. It is a fairly well differentiated adenocarcinoma and neither invasion nor metastasis has been observed. All attempts to transfer the tumor to other mouse strains have been unsuccessful, and in no instance has growth been obtained in the anterior chamber of the guinea pig's eye.

Rat Sarcoma 39

This tumor originated as a fibroadenoma in the mamma of an old female market rat in the Department of Cancer Research in Columbia University, but its subsequent development was that of a polymorphous cell sarcoma, and it has been propagated there as such for many years. Takes occur in from 50 to 100 per cent of the Wistar strain of rats used, and approximately 30 per cent of the resulting tumors regress. The tumor has been successfully transferred to several varieties of rats in our laboratory, but in all these the eventual fate of the growth was regression. The mode of growth is predominantly expansive but invasion does occur.

Transfer to the anterior chamber of guinea pig eyes.—Transfer to guinea pig eyes is successful in approximately 50 per cent of cases. Growth is extremely rapid and apparently proceeds for a time in the manner of a tissue culture, for the chamber may be one-third filled with tumor before the appearance of a vascular supply. This is particularly evident when small particles become detached from the main fragment during passage through the chamber and give rise to multiple isolated foci of growth. Here the growth of individual cell clumps can be easily followed with the aid of a magnifying lens, and enlargement in all directions to a rounded translucent mass precedes penetration by blood vessels. After vascularization the growth rate is further increased, and the chamber may be completely filled by the sixth day. The appearance of large hemorrhagic areas in the substance of the transplant, and of free blood in the chamber, is a constant feature of growth after vascularization and obscures the sequence of later events. Eventually, however, after a lapse of several weeks regression occurs. It may be interrupted by a period of renewed growth, but ultimately becomes complete. The eye is extensively damaged and after healing presents the picture of phthisis bulbi.

Histological examination of the transplants after vascularization shows extensive invasion of the iris, with growth along the choroid and consequent dislocation of the retina. Tumor cells are found both in large mass formations and as single isolated units throughout the eye. Morphologically the appearance and arrangement of the cells suggest a carcinomatous rather than a sarcomatous character and resemble the growing edge rather than the main bulk of the rat tumor (Figs. 26 and 27).

Transfer to the anterior chamber of rabbit eyes.—Transplants of the tumor grow in 40 per cent of

DESCRIPTION OF FIGS. 19 TO 24

Fig. 19.—Transplant of hepatoma growing in subcutaneous tissues of Bagg albino mouse. Mag. × 275.

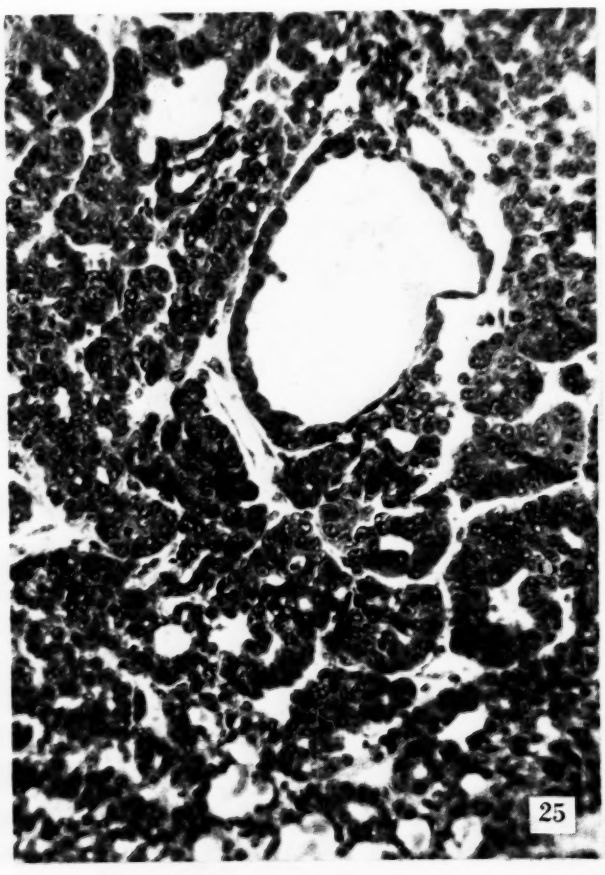
Fig. 20.—Transplant of hepatoma growing in anterior chamber of a guinea pig's eye. Mag. × 275.

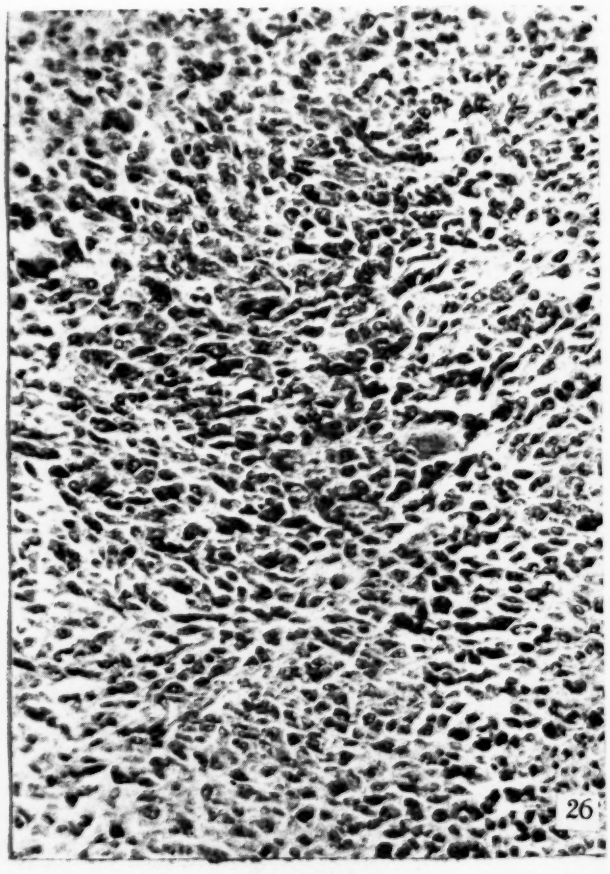
Fig. 21.—Transplant of mammary carcinoma RC growing in subcutaneous tissues of dba mouse. Mag. × 275.

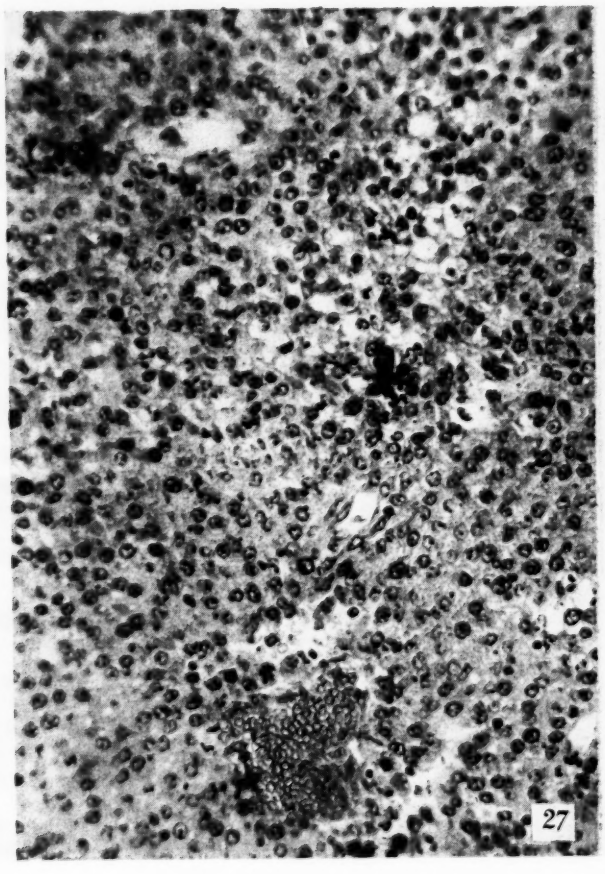
Fig. 22.—Transplant of mammary carcinoma RC growing in anterior chamber of guinea pig's eye. Mag. × 275.

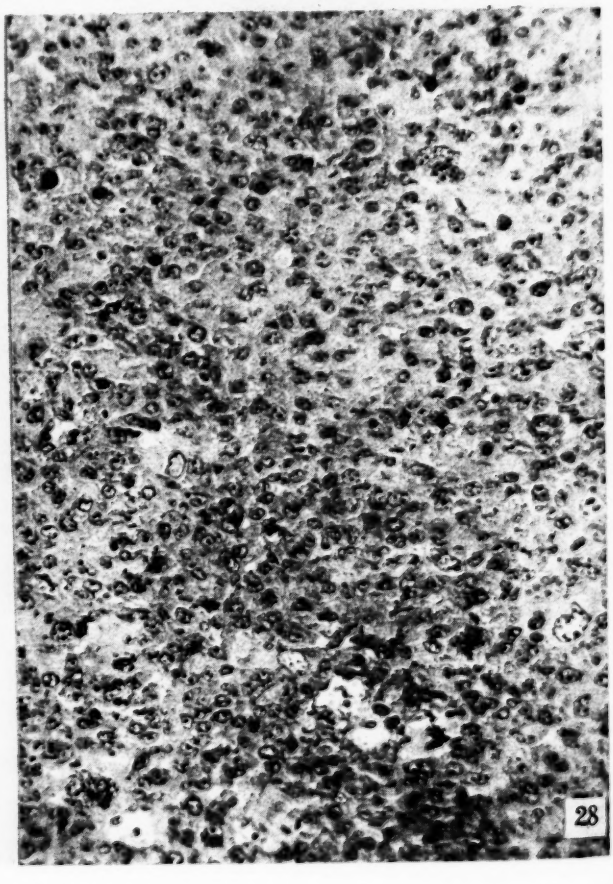
Fig. 23.—Transplant of mammary carcinoma RC growing in anterior chamber of rat's eye. Mag. × 275.

Fig. 24.—Transplant of Yale Tumor No. 1 growing in subcutaneous tissues of C3H mouse. Mag. × 275.









FIGS. 25-28

rabbits, and their behavior is comparable to that described in guinea pigs. Histologically the resemblance to carcinoma is even more pronounced (Fig. 28).

Rat Mammary Tumor 2426

This tumor arose in the mamma of a female rat and the characteristics of the growth in the primary host and in experimental animals have been described (1). It is a highly differentiated growth and, according to Eisen, does not metastasize. In our laboratory neither invasion nor metastasis has been observed.

Attempts to transplant the tumor subcutaneously into rats of several different strains have been unsuccessful, and in no instance has growth been obtained in the guinea pig.

Return to the Mouse after Guinea Pig Passage

After growth for a generation or more in the anterior chamber of the guinea pig's eye, the tumors have been transferred back to the subcutaneous tissues of mice of the strain in which they originated, and without exception the speed of growth, power to invade, and metastatic rate are increased. Thus when the bronchogenic carcinoma is returned to the abdominal subcutaneous tissues of C3H mice, takes are apparent within 2 days. Expansive growth is reduced to a minimum, and in contrast to the local swelling characteristic of ordinary subcutaneous transplants the tumor appears as a diffuse, plaque-like thickening of the greater part of the abdominal musculature, resembling in many respects the growth of a lymphosarcoma transplanted to the same region. Invasion of the peritoneal cavity occurs with great rapidity, and the animals frequently die with lung metastases in less than 2 weeks after transfer.

DISCUSSION

The ability of some human and rabbit tumors to survive and to grow in animals of alien species has been demonstrated, and it is apparent from the experiments described in the present paper that this attribute also distinguishes certain tumors of the mouse and rat. The property of heterotransplantability is not shared by all tumors of these species but appears, on the other hand, to be a common characteristic of a special group, and thus offers a means of classification based on biological behavior. The significance of such a classification is the subject of present investigation, but the evidence already at hand is highly suggestive and warrants consideration.

In all the species studied the ability to survive heterologous transfer is restricted to a group of tumors characterized by distinctive properties with respect to manner and range of growth in the parent species. In the rabbit the heterotransplantable tumors, in contrast to those not transferable in this manner, are distinguished by the capacity to invade and metastasize and to grow in unrelated animals of the same species. Similarly, in man, only those tumors that invade and metastasize are capable of growth in alien species, but whether or not they possess the power to grow in unrelated individuals of the same species is obviously a question not amenable to experimentation. An examination of the neoplasms utilized in the present investigation reveals a situation comparable with that observed in the rabbit—that is, the tumors that invade and metastasize are transplantable to unrelated strains and to alien species, whereas those that show neither invasion nor metastasis in the parent stock fail to grow when transferred to unrelated strains or to foreign species.

Thus the ability to invade and to metastasize characterizes the heterotransplantable tumors of all the species studied. This same ability is apparently an essential property of neoplasms that are capable of survival and growth in unrelated strains of the parent species. Tumors that invade and metastasize can be transferred both to unrelated animals of the same species and to animals of entirely unrelated species, and there is no evidence to indicate that the two types of transfer reflect different biological potencies. On the contrary, it is suggested that heterotransplantability does not imply the assumption of new and different properties on the part of the tumor, but that the attributes allowing true homologous transfer also allow heterologous transfer.

It is essential in the present connection to emphasize the biological distinction between successful transfer to animals of the same strain and successful transfer to animals of an unrelated strain. All mouse tumors appear to be transplantable in animals of the strain in which they originate. But this does not constitute homologous transfer, for as a result of long inbreeding the donor and the recipient in such an experiment bear a genetic relationship somewhat comparable to that of the fore and hind quarters of the same individual. Successful transfer of this type, therefore, does

DESCRIPTION OF FIGS. 25 TO 28

Fig. 25.—Transplant of Yale Tumor No. 1 growing in anterior chamber of guinea pig's eye. Mag. × 275.

Fig. 26.—Transplant of rat sarcoma 39 growing in the subcutaneous tissues of a hybrid rat. Mag. × 275.

Fig. 27.—Transplant of rat sarcoma 39 growing in anterior chamber of guinea pig's eye. Mag. × 275.

Fig. 28.—Transplant of rat sarcoma 39 growing in anterior chamber of rabbit's eye. Mag. × 275.

not attain the same biological significance as homologous transfer in species such as the rabbit, where inbreeding has not been practiced. In effect, such transfer in the mouse is comparable to autologous transfer in the rabbit, and just as all mouse tumors grow in related animals so, also, all rabbit tumors grow on autologous transfer. Homologous transfer in the rabbit is possible only after progressive development of the tumor, and in terms of biological behavior marks the distinction between dependency and autonomy or, in clinical terms, between benignancy and cancer. It seems probable that a similar situation obtains in the mouse, and that with respect to autonomy successful transfer within a strain is of no greater significance than autologous transfer in the rabbit.

It is suggested that at this stage of development, when only limited transfer is possible, the tumors are dependent for continued survival and growth on factors peculiar to the primary host and closely related individuals. These factors are not supplied by unrelated animals, and such animals will not support growth of the tumors on transfer. At a later stage autonomy is attained, the tumors become independent of the factors concerned in this development, and gain the ability to survive and grow in their absence. The independence thus achieved eliminates genetic and species barriers and allows growth in unrelated animals and in alien species.

The tumors employed in the present study may be classified with these points in mind. On such a basis the dependent growths are the mammary tumors 755, BR, ST, and 2426, while the autonomous growths are the bronchogenic carcinoma, 180, Yale 1, RC, the embryoma, the hepatoma, and R39.

It has been found by means of successive biopsies and transplantation experiments, performed throughout the developmental course of cancer in rabbits, that the tumors undergo phases of dependency and autonomy and that the attainment of autonomy is only evidenced by invasion and metastasis (6). Comparable experiments utilizing spontaneous mouse tumors are in progress, and will be reported in detail in a later paper. However, for present purposes it may be noted that the developmental course of tumors in this species is also characterized by dependent and autonomous phases. During early or dependent stages the growths are transplantable only into animals of the same strain, but after continued development with invasion and metastasis they become transplantable in unrelated strains and in alien species. On this basis it seems probable that the dependent tumors in the series under study were transferred from the primary host before development had been completed. On the other hand, the tumors found to be autonomous may have been transferred at a later period in their developmental course, after the attainment of independence or, conceivably, during a dependent phase with later inadvertent transfer to animals supplying factors necessary for their continued development to cancer.

In any case, it is clear that the mouse and rat tumors studied are divisible into two classes on a basis of fundamental differences in biological behavior, and that tumors of the different groups can not be used as comparable materials in cancer research.

SUMMARY

A number of mouse and rat tumors including a bronchogenic carcinoma, sarcoma 180, an ovarian embryoma, an experimentally induced hepatoma, 2 mammary carcinomas, and sarcoma 39 were successfully transplanted to animals of alien species. All the heterotransplantable tumors, in contrast to a group not transferable in this manner, possessed the ability to invade and metastasize in the parent strain and to survive and grow in unrelated strains. On this basis it is concluded: first, that in mice, as well as in man and in the rabbit, invasion marks the attainment of autonomy; and second, that from the point of view of autonomy true homologous transfer and heterologous transfer possess the same significance.

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Thymonucleic Acid in Tumors*†

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OUTLINE

INTRODUCTION

The vital activities of cells are controlled to a great extent by complex conjugated proteins, nucleoproteins, which consist of one or more protein molecules combined with nucleic acids. These nucleic acids are esters of phosphoric acid and glucosides that consist of a pentose sugar (ribose or desoxyribose) and a cyclic derivative (adenine, guanine, cytosine, thymine, or uracil). The desoxyribonucleic, or thymonucleic acid, is found in the nuclear chromatin and the ribonucleic, or yeast nucleic acid, chiefly in the cytoplasm of plant and animal cells (5, 15, 65, 89, 90, 94).

Only a satisfactory beginning has been made toward attaining a complete understanding of the nucleoproteins. With new research methods progress will be more rapid, and the present views will probably be altered according to subsequent findings. Following the increasing recognition of the importance of nucleic acids in normal cells, evidence is accumulating to suggest that there is a disturbance of the normal balance of nucleic acids in some tumors. This paper will summarize the pertinent results of the investigations of thymonucleic acid in normal cells, and the evidence that it may play a significant role in tumors. Although the evidence is still inadequate to determine whether the observed changes in nucleic acid represent a primary cause, a contributing cause, or a result of neoplasia, it is hoped that the present summation, tentative though some of the views must be, will serve as a stimulus to increased discussion and investigation.

Certain aspects of nucleic acids will not be taken up in this paper. Their chemical and physical properties have been described by Levene and Bass (76) and by others (20, 48, 49, 65, 67, 99, 104). Physiological effects of the injection of nucleic acid have been described, such as an initial leukopenia followed by leukocytosis (50, 55, 59, 93, 97) and a lowering of the alkaline reserve and increase in inorganic phosphorus and sugar of the blood (55).

Extracts of cells that contain nucleic acids and nucleotides have a growth-promoting effect on other cells. Tennant, Stern, and Liebow (123) reported a stimulating effect upon the growth of mouse heart fibroblasts *in vitro* by various nucleic acids, including thymus thymonucleinates. The extraction of embryonic and adult tissues yields a substance that promotes the growth of tissues *in vitro* (47, 57, 58, 119, 122). These extracts contain varying proportions of the ribose and desoxyribose types of nucleic acids. Usually the ribonucleic acid has more stimulating action than the desoxyribose type. Saha and Ghosh (97) concluded that nucleic acid had no growth-promoting value in rats.

The growth-stimulating action of material from injured yeast cells has been attributed by Loofbourow (79, 80) to adenine nucleotides rather than yeast adenylic acid or yeast nucleic acid. However, using similar material, other workers (37) conclude that the nucleosides, adenosine and guanosine, alone or combined with each other or with yeast adenylic acid, are important active constituents. Davidson (45) recently reviewed the subject of wound hormones and suggested that ribonucleic acid may be an important constituent of some of these substances that cause proliferation of cells. The possible relationship to neoplasia of such wound hormones, containing nucleic acids or their constituents, is an interesting sub-

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ject for speculation, but one that lacks substantial experimental evidence.

The Role of Thymonucleic Acid in Normal Cells

According to generally accepted ideas, nucleoproteins control the hereditary and vital functions of living cells. The most important nucleoprotein within the nucleus is the desoxyribose tetranucleotide, which is combined with basic proteins of the protamine and histone type (23, 89). Although the nucleic acids of a nucleoprotein are usually one of two types, the protein may vary from one species to another and may be relatively specific. The thymonucleic acid has important roles in controlling heredity, in mitosis, in polymerization of nucleoproteins, and in the formation of proteins and of ribonucleic acid of the nucleolus and cytoplasm. In leukemic blood cells the thymonucleic acid comprises 40 per cent of the chromatin complex (36).

Recently Stedman and Stedman (108-111) have presented evidence that a protein, which they have named chromosomin, is the most important constituent of chromosomes and the chemical basis of inheritance. They suggest that nucleic acid is united to this protein. The views of Stedman and Stedman that the Feulgen reaction is not a specific stain for thymonucleic acid, and that this acid is a more important constituent of the nuclear sap than of the chromosomes, have been severely criticized (3, 19, 26). It is asserted that Stedman and Stedman have misconstrued the work of other investigators and presented insufficient proof to substantiate their own statements. The possibility of the existence of a protein such as chromosomin may be admitted, but a final evaluation of its significance must await the publication of additional evidence.

Some cells seem to contain chiefly pentosenucleic acids of the ribose type and very little desoxyribonucleic acid. This is especially noticeable in some ova (17) and in protozoa. In such cells the nucleoproteins may undergo cyclic variations in which, at some stages, the desoxyribonucleic acid is at a minimum. Thus Meglitsch (87) has shown that in the life cycle of the protozoan *Endamoeba blattae* there are two types of cyclic variation in the amount of thymonucleic acid, one associated with division of the trophic amebae and the other with encystment.

Chromosomes and genes.—The chromosomes are enormously extensible protein fibers to which is attached desoxyribonucleic acid at certain specific points, the genes (42, 101).

There are two types of regions in the chromosomes; the active genes, or euchromatic regions, and the inactive, or heterochromatic, regions. The inert chromatin of the heterochromatic regions retains an extra charge of nucleic acid between metaphases. Underlying the cycle of mitosis and cell division is a concomitant cycle of attachment and detachment of this nucleic acid to and from the chromosomes, which accompanies the coiling and uncoiling of their protein framework. According to Caspersson (27) the maximum attachment corresponds with the maximum spiralization of the chromosomes at the metaphase. On the other hand, they are uncoiled and relatively free from thymonucleic acid within the resting nucleus.

At the end of mitosis the chromosomes give up their nucleic acid charge and secrete nucleoli, which dissolve at the initiation of the next mitosis when the nucleic acid content of the chromosomes is increased. Most nucleoli contain only ribonucleic acid, although Eckert and Cooper (56) and Koller (74) have reported desoxyribonucleic acid in the nucleoli of some tumor cells.

By using ultraviolet spectroscopy in combination with other methods (21, 23), Caspersson (24, 27) has been able to differentiate qualitatively and quantitatively between the 2 nucleic acids or their nucleotides, which have a high maximum absorption located at 2,600 A., and the proteins, which have a lower maximum absorption at 2,750 to 2,900 A. He has provisionally identified a histone and a globulin type of protein. The heterochromatin and nucleolus both show a high content of histone, whereas the euchromatin between the heterochromatic regions contains proteins of the globulin type. In the chromosomes at metaphase these higher globulin proteins are absent, and only histones and thymonucleic acid are recognizable. Caspersson believes that the active genes in the resting nucleus are producing large molecules of globulin while the inactive ones are producing smaller molecules of the histone type, which are less specific in their interactions. Thus the difference between activity and inertness of chromatin is a difference between high and low specificity of proteins as well as high and low content of nucleic acid. The interphase chromosome is dominated by the characteristics of the protein, and the mitotic chromosome by the nucleoprotein.

The similarity of content of the heterochromatin and nucleolus suggests that the heterochromatin secretes the nucleolus or a precursor of it. This belief is supported by the common observation that the size of nucleoli is proportionally greater in cells which, with the exception of nerve cells, are concerned with the most rapid protein production, and smallest in cells where no protein is being formed. Thus it is significant that ribonucleotide increases in concentration as cells produce increased amounts of proteins. The ribonucleic acids are constructed near

the nuclear membrane where the nuclear and cytoplasmic surfaces meet (Caspersson and Schultz, 31).

Cytoplasmic nucleic acids.—Because of the interrelationships of desoxyribo- and ribonucleic acids it is not possible to consider the nucleoproteins of the nucleus exclusive of the nucleic acids of the cytoplasm. The nucleoproteins important in cytoplasmic synthesis are influenced by genetic changes in the chromosomes (101). An increase of heterochromatin in the nucleus increases the concentration of cytoplasmic nucleic acids (Caspersson and Schultz, 29).

Yeast contains about 5 to 10 per cent and some bacteria 15 to 20 per cent of their dry weight of ribonucleic acid (Mirsky, 88). The pancreas contains 10 per cent of its dry weight of ribonucleic acid, which is more than is found in any other animal tissues.

Rapidly dividing cells have high concentrations of desoxyribo- and especially of ribonucleic acid, whereas the absorption band of mature cells shows that they consist chiefly of proteins. In the cytoplasm of cells in the process of oogenesis—a cytoplasm in which many divisions are to occur—Brachet (16) has shown that the ribonucleic acids decrease while the desoxyribonucleic acids increase in amount. The distribution of ribonucleic acids in cytoplasm parallels the synthetic activities that are proceeding there. The basophilic staining of glands and embryonic tissues has been attributed to the high concentration of nucleic acids associated with rapid protein synthesis.

There is some evidence that the cytoplasmic nucleic acids have a higher rate of phosphorus turnover than the nucleic acids of the nucleus. In the resting state, the continuous turnover of nucleic acids is very slow, whereas synthesis takes place rapidly during growth (Brues, Tracy, and Cohn, 18). Although the relative amounts of nucleic acids may be considerably increased in embryonic tissues, the observations of Davidson and Waymouth (46) indicate that the amount of ribonucleic acid relative to the amount of thymonucleic acid remains the same, or may be slightly greater, in the adult as compared with that in the embryo. Claude (32-35) has used a differential centrifugation technic to isolate particulate components of normal tissues and tumors. These small cytoplasmic bodies, which Claude considers as mitochondria, are aggregations of ribonucleoproteins associated with various proportions of phospholipids. The possible role of mitochondria in the synthesis or storage of ribonucleic acid is not understood. Because of their wide distribution, the presence of ribonucleoproteins in tumors is of significance only if it is demonstrated that their amounts of configuration are unusual.

Protein synthesis.—The nucleus is the center of protein synthesis in the cell, and the nucleic acids are the essential agents of this synthesis. The desoxyribonucleic acids may be important not only for the formation of a chain structure by their own polymerization, but also for their actual synthesis of the fibrous proteins of the chromosomes (Schultz, 100).

Caspersson (27) has developed the hypothesis that the process of protein synthesis proceeds by way of the histones in the nucleolus, which diffuse through the nuclear membrane forming the ribonucleic acids concerned with the synthesis of cytoplasmic proteins. Thus the special heterochromatic regions concerned with the formation of the nucleolus are the major producers of histones.

The ribo- and desoxyribonucleic acids are similar. They seem to bear a reciprocal relationship to each other, and can probably be formed interchangeably from each other during the cyclic variations of nucleic acid within the cell. However, the desoxyribose radical apparently gives its nucleotides a flatness to which they owe their capacity for polymerization. In the native state the desoxyribonucleic acids may form polymers with a molecular weight of as much as 1,000,000 (Signer, Caspersson, and Hammarsten, 104). These desoxyribonucleotides form columns of plates, which Schmidt (99) has shown by polarized light to lie crosswise to the protein thread. According to Caspersson this orientation is somewhat less precise. These plates agree in spacing with the location of the side chains on the extended polypeptide chain of the chromosome. Thus the possibility for chain formation exists in both the protein and in the prosthetic nucleic acid group (Schultz, 100). Because of this capacity thymonucleic acid is considered essential in the reproduction of the chromomere. Such a system of polypeptide chains, with their numerous and varied side chains and their ability to change shape by intramolecular folding, would appear to be well suited to the genetic task of bearing the patterns of life (Astbury, 1).

Bacteria and filterable viruses.—Bacteria contain nucleic acids of the ribose, desoxyribose, and, in the case of tubercle bacillus, perhaps a third type (76, p. 277). Of the total nucleic acid in streptococci, 10 to 30 per cent is of the desoxyribose type and the remainder of the ribose type. Two to 6 per cent of the dry weight of bacteria is thymonucleic acid (Sevag, Smolens, and Luckman, 102). Henry and Stacey (68) have suggested that the gram-positive material in bacteria is a high-molecular complex formed by the combination of a reduced basic protein substrate with magnesium ribonucleate.

The work of Avery and his co-workers (2) gives some interesting information on the role of thymonucleic acid in bacteria and in heredity. The most striking example of inheritable and specific alterations

in cell structures that can be experimentally induced and reproduced among bacteria is the transformation of specific types of pneumoccoci. A biologically active fraction was isolated in highly purified form from type III pneumoccocus that was capable of inducing the transformation of unencapsulated R variants of pneumoccous type II into encapsulated cells of the same specific type as that of the heat-killed bacteria from which the inducing material was removed. The material consisted of a highly polymerized viscous form of desoxyribonucleic acid. The alterations produced were predictable, type specific, and transmissible in series. The type III capsular substance that is evolved by the nucleic acid is a non-nitrogenous polysaccharide and quite different from thymonucleic acid. This work with desoxyribonucleic acid is one of the first successful attempts to induce, by chemical means, predictable and specific changes that are transmissible thereafter in series as a hereditary character.

Chemical analyses of both plant and animal viruses show that they all contain some nucleoprotein, and some of the smaller viruses are pure nucleoprotein (Stanley, 105, 106). The nucleoproteins of the plant viruses are of the ribose type (75, 107). Loring (81, 82) found evidence that the pentosenucleic acid of tobacco mosaic virus differed from the usual yeast nucleic acid. Tobacco ringspot virus is 40 per cent nucleic acid and 60 per cent protein.

The findings in animal viruses are variable. These, in contrast to plant viruses, usually contain some lipid or phospholipid. The desoxyribose type of nucleic acid has been found in some animal viruses. According to Hoagland, Lavin, Smadel, and Rivers (70) the elementary bodies of vaccinia contain at least 5.6 per cent thymonucleic acid and possibly a small amount of ribonucleic acid. Psittacosis virus also contains thymonucleic acid. The rabbit papilloma virus contains 8.7 per cent of thymonucleic acid, 6.5 per cent carbohydrate, and 1.5 per cent lipid, with no evidence of ribonucleic acid (118). The virus of the eastern strain of equine encephalomyelitis is a complex of high molecular weight consisting of 54 per cent lipids, and ribonucleoprotein, of which 10 per cent was ribonucleic acid (103, 120). Nucleic acid of the desoxypentose type has been identified in swine influenza virus (121).

Stanley believes that the viruses have essentially the structure of genes but are further adapted for independent existence. However, the genes themselves contain chiefly the desoxyribose type of nucleic acid and have little if any ribonucleic acid. Both genes and viruses mutate (101). The suggestion has been made by Pollister and Mirsky (94) that certain viruses may be related to the self-duplicating bodies of the nucleus, the genes, which contain desoxyribonucleoproteins, and that other viruses may be related to the self-duplicating bodies in the cytoplasm such as the chloroplasts and mitochrondria, which contain ribonucleoproteins (35). The presence of nucleic acids in viruses and phages and the augmentation of nucleic acids on the chromosomes at the time of reproduction of the genes support the idea that it seems to be characteristic of self-producing substances to have nucleic acids in their molecules, at least during the time of their reproduction.

The Role of Thymonucleic Acid in Neoplastic Cells

Experimental cancer research has shown that the concept of "*the* cause of cancer" is now obsolete. Because of the complex and variable nature of neoplasia a single cancer may be attributable to several factors. Histologically identical cancer may be elicited by several quite different etiologic agents. Under controlled laboratory conditions the Shope papilloma virus, any one of several synthetic carcinogens, and ultraviolet light may produce epidermoid carcinomas of the skin that are indistinguishable. Mammary tumor-inciting substances, estrogenic stimulation, and hereditary constitution may contribute simultaneously as causes of a mammary cancer in a mouse.

The causes of cancer may be divided into the extracellular and the intracellular. In the study of extracellular causes oncologists have made considerable progress. It is recognized that factors such as chemical carcinogenic agents, heredity, hormones, milk factors, viruses, physical trauma, and precancerous conditions may be contributory causes of cancer in the lower animals and in man. In the study of intracellular causes, however, progress has been much less satisfactory. The nature of the intracellular change that characterizes neoplasia is unknown, and although a knowledge of the extracellular causes may permit us to prevent and to cure cancer, it will be necessary to know the intracellular causes in order ultimately to understand and control it.

What is the nature of the intracellular changes produced by various extracellular carcinogenic factors such as viruses or chemical carcinogens, which, after a variable latent period, cause subsequent generations of cells to exhibit the characteristics of malignancy? Although the answer to this question is not yet known, several of the theories that have been advanced as explanations of the change will be examined briefly.

One of the more popular theories of etiology is the virus theory. That some tumors such as the Rous fowl sarcoma are elicited by a virus is generally accepted, but there is inadequate evidence at present that any large proportion of tumors are so caused. The proponents of the virus theory have received some

support from the recent work on the milk factor, which apparently has many properties of a virus.

As already mentioned, the nucleic acids are probably the most important constituents of viruses. A desoxyribonucleic acid capable of inducing a cellular alteration transmissible to subsequent generations of bacteria has been discussed. It is possible that the nucleic acids of viruses might also induce transmissible intracellular changes characteristic of neoplasia. Potter (95), in his discussion of an enzyme-virus theory of carcinogenesis, mentions the possible significance of ribonucleoprotein in the production of cancer. However, the demonstration of a virus as an etiological factor of a tumor does not explain the nature of the intracellular change associated with cancer any better than the observation that synthetic carcinogens elicit neoplasia.

Rhoads (96) and his colleagues showed that a metabolite of *p*-dimethylaminoazobenzene poisoned the coenzyme I system of the normal liver cell, and that cancer cells may develop a resistance to this toxic agent. From such research an enzyme theory of etiology has been evolved, suggesting that because of an interference with its enzyme system a cell is forced to develop a new type of metabolism that is not subject to the usual regulatory mechanism of the organism. At present there is insufficient evidence to recognize the importance of such an enzymatic disturbance as a general cause of cancer, although this is one of the more interesting fields of investigation.

Loeb (78) has suggested that the intracellular cause of cancer is a series of progressive changes accompanied by the formation of excessive amounts of autocatalytic growth substances. The evidence suggesting that nucleoproteins and their nucleic acids may be important in such autocatalytic action will be presented in this review.

The significance of nucleic acids in the somatic mutation theory of etiology.—One theory that does attempt to explain the intracellular change in many tumors is that of somatic mutation. Its historical background has been reviewed by Bauer (4), and more recently by Berrill (6) and by Furth, Boon, and Kaliss (60). Boveri (14), who is usually credited with originating the idea that cancer is due to a definite abnormal chromosome complex, traced it to von Hansemann. Although the theory has been discussed by many writers, with but slight modification, conclusive evidence of a change within the chromosomes has not been presented. The best evidence has been advanced by Biesele and his associates (7-13), who found an increase in number and size of the chromosomes in many tumor cells. The investigations of Heston (69) on genetic factors of susceptibility of mice to pulmonary tumors is one piece of experimental evidence that lends support to the idea of the localization of susceptibility to cancer in a single definite gene. He found that heterozygous mice bearing the lethal A^y gene for yellow coat color had a higher incidence of induced pulmonary tumors than control mice.

The objection is sometimes raised that mutations are believed to be relatively rare while cancer is a more common occurrence. However, conditions in the mammalian body may be extremely sensitive for the disclosure of a cancer cell. For example, if one of the billions of normal lymphocytes in the mammalian body assumes malignant properties, the organism will develop leukemia (88).

When Hollaender, Greenstein, and Jenrette (63, 71) exposed aqueous solutions of sodium thymonucleate to radiation of 2,537 A. they observed a loss of structural viscosity and streaming birefringence due to a progressive depolymerization of the thymonucleate. From the genetic and physiologic viewpoints, it is interesting to speculate on the processes involved in the effects of ultraviolet radiation. The wave length range near 2,600 A. is most effective for lethal action and gene mutation. The nucleic acids of the cells are readily affected by ultraviolet light. The chromosomal changes that result in mutations may be initiated by an alteration or breaking down of the nucleic acid macromolecules. Thus it has been demonstrated that the physical agent, ultraviolet light, which is known to be active in the production of genetic mutations in lower organisms and in eliciting cancer in mice, produces a breaking down of thymonucleate. Although the proof is not at hand, it is conceivable that some of the other factors that aid in carcinogenesis might also exert their action through the induction of a transmissible change in the nuclear proteins. A disturbance in either the nucleic acid or protein component would produce effects upon the other constituents of the nucleoprotein.

According to Lockhart-Mummery (77), the only recognizable and important form of gene mutation occurring in a somatic cell would consist of, or be accompanied by, an increased rate of division as compared with the normal rate for that cell. In this way a colony of mutant cells, a tumor, would be produced among the normal cells.

Donovan and Woodhouse (51) have suggested that an unorthodox production of nucleotides or nucleic acids is the basis for the abnormal growth of tumors. Some of the details of the chemistry involved in their theory, such as an improbable stereochemical configuration and composition of nucleic acid molecules, have been justly criticized by Gulland, Barker, and Jordan (66).

The importance of the thymonucleic acids in the hereditary and vital functions of normal cells has

been discussed. If the somatic mutation theory is correct, it seems probable that such changes would involve the desoxyribonucleotides of the nucleus. That there is a disturbance of nucleic acids in cancer has been suggested by the experimental observations to be discussed in the following sections.

Chemical evidence of disturbance of nucleic acids in tumors.—The results of chemical analyses of nucleoproteins by various workers are unsatisfactory in some instances, and contradictory in others. The reports of increased nucleic phosphorus and diminished lipid-phosphorus in malignant tumors, as compared with normal tissues, may be explained by the increased content of nuclei in malignant growths (113, p. 129). The few investigations in which there was a chemical isolation and quantitative analysis of nucleic acid from tumors did not give conclusive results. In nucleic acids isolated from metastases to the liver, the nitrogen content averaged 9.8 per cent and the phosphorus-nitrogen ratio was 1:1.2 to 1:1.0 as compared with a nitrogen content of 14 to 16 per cent and a phosphorus-nitrogen ratio of 1:1.76 in normal tissues (112, 125). Other investigators, including Klein and Beck (73), were unable to confirm a diminished nitrogen content of tumor nucleic acids.

Greenstein, Jenrette, and White (64) concluded on the basis of chemical analyses that the corresponding nucleoprotein fractions from rat liver and from transplanted hepatic tumors were nearly identical. The fact that the nucleoproteins are similar does not exclude the possibility that their configurations might be different.

Dounce (52-54) has extracted the desoxyribonucleic acid from normal rat liver, rat hepatoma 31, and Walker rat carcinosarcoma 256 by chemical methods. He concluded that the nuclei of Walker tumors have nearly the same concentration of desoxyribonucleic acid as the nuclei of normal rat liver, while the nuclei of hepatoma 31 appear to have a much lower desoxyribonucleic acid content. His observations were based on the percentage of dry weight of nuclei and are not directly referable to such important biologic criteria as the mean amount of nucleic acid per unit volume of tissue, per unit volume of nucleus, or per nucleus. Variations in size and water content of tumor cells are important factors. The extraction of nucleic acid from tissue is a technical procedure that needs improvement before being generally accepted as a highly accurate quantitative procedure.

Qualitative color reactions revealed no change in the carbohydrate content of nucleic acids of tumors as compared with normal tissues (73, 125).

Masayama and Yokoyama (86), using a colorimetric macrochemical technic, reported an increase in thymonucleic acid in the livers of rats that had been fed *p*-dimethylaminoazobenzene. The nucleic acid was doubled after 30 days, and there was a sudden further increase as cancer developed.

Both the ribo- and desoxyribonucleic acids occur in polymerized forms, with molecular weights estimated to be about 20,000 for the former and 1,000,000 for the latter (67, 81). In this form the yeast nucleic acid is relatively insoluble in acids, and the sodium salt of thymonucleic acid shows streaming birefringence and structural viscosity. When the nucleic acids are treated with tissue enzymes from various sources, these specific physical properties are progressively diminished. The depolymerase for yeast nucleic acid, ribonucleodepolymerase, is heat-stable, while the depolymerase for thymonucleic acid, desoxyribonucleodepolymerase, is heat-labile.

Greenstein and his co-workers (61, 62), in their extensive studies on enzymes in tumors, tested these 2 depolymerases on milk, and on several tumors and the corresponding normal adult and embryonic tissues. Since their results showed variations from one type of tumor to another, and from similar tumors in different species of animals, it is difficult to correlate their findings with other observations. The 2 depolymerases do show a striking and parallel behavior concerning their content in various tissues.

Histochemical and cytochemical evidence of disturbance of thymonucleic acid in tumors.—Histochemistry is the study of the chemical constitution of tissues, whereas cytochemistry deals with the chemical constitution of the elements of a cell. Numerous workers have made visual observations on cancer cells stained by the Feulgen technic. Cowdry (38) compared tissue cultures of rat sarcomas and of normal fibroblasts, and reported an increased amount of Feulgen-stained material in the nuclei of sarcoma cells. On the other hand, Ludford (83) found no difference in the amounts of thymonucleic acid in the nuclei of a tar tumor as compared with the surrounding skin. He was unable to demonstrate any relation between the amount of chromatin in the nucleus of a tumor cell and the rate of growth of the tumor. Eckert and Cooper (56), studying epidermoid carcinoma of the cervix, did not observe any significant differences in the amount of Feulgen-stained material in malignant and normal cells.

As a part of the research project on experimental carcinogenesis of the skin under the direction of Dr. E. V. Cowdry (39, 40), Stowell determined the relative thymonucleic acid content of normal, hyperplastic, and neoplastic epidermis of mice (114). He employed a photometric histochemical method in which the relative absorption of monochromatic light in sections of tissues stained by the Feulgen reaction

for thymonucleic acid was determined by a special microphotometer.

With the same methods similar measurements were made on biopsy material from patients with epidermoid carcinoma of the skin (117), and results similar to those for carcinoma of the skin in mice were obtained. The order of decreasing mean amounts of thymonucleic acid per unit volume of tissue and per cell was carcinoma > normal > hyperplastic epidermis. Half of the carcinomas contained increased amounts of thymonucleic acid per cell that were statistically significant and in no instance was it significantly decreased. Additional investigations will be necessary to determine the reason for the variations in the increase in the relative amount of thymonucleic acid in specific tumors.

Preliminary investigations by Stowell (115) have shown that the amount of thymonucleic acid is increased in the leukemic cells of patients with lymphoid leukemia. There is some evidence that irradiation produces a disturbance in the balance of nucleic acid of malignant cells, accompanied by a decrease in the amount of desoxyribonucleic acid (116) in the nucleus and an increase in the amount of ribonucleic acid (91) in the cytoplasm.

Caspersson, using cytochemical methods, concluded that the heterochromatic section of the nucleus plays a specific role in carcinogenesis (Caspersson and Santesson, 28; and Koller, 74, p. 246). In a study of human carcinoma Caspersson (27, 28) found that the cytoplasm of tumor cells contained larger amounts of ribonucleic acid than corresponding normal cells. The amounts varied in different parts of the same tumor, and were larger in areas of more rapid growth. In view of the large amounts of ribonucleotides in the cytoplasm of rapidly growing normal cells Caspersson and Schultz (30) believe that the vigorous growth character of the malignant cell is evidenced by the large nucleoli, which play an important role in protein synthesis. It is a recognized fact that large nucleoli are characteristic of malignant cells (41, 85).

Mitchell (91), using ultraviolet absorption for nucleic acids, did not observe any significant difference between normal and hyperplastic tissues and malignant tumors, but relatively few cells were measured in each specimen.

Cytological evidence of abnormalities of nucleic acids in chromosomes and nucleoli in tumors.—Koller (74), who carried out a cytological analysis on 565 neoplasms of the human skin, esophagus, colon, rectum, larynx, lung, cervix, uterus, and breast, presents interesting ideas regarding the nature of the disturbed nucleic acid metabolism in tumors. It was found that nucleoli in cells of the same tumor, as well as in separate tumors, differed in their chemical content and in their size. The small, deep-staining nucleoli represented heterochromatic regions in the chromosomes that retained the desoxyribonucleic acid during the resting stage. The larger nucleoli, which varied greatly in size, contained large amounts of ribonucleic acid and histone. The proportions of these two types of nucleoli varied in the same and in different tumors. With the development and growth of the neoplasm the proportion of Feulgen-negative nucleoli containing ribonucleic acid increased in proportion to other cells.

Abnormalities of mitosis were expressed in chromosome structure and behavior, and in lack of coordination between spindle mechanism and chromosome movements. A close relationship was present between the frequency of abnormalities and the type of nucleolus. The tumors with few Feulgen-negative nucleoli had few abnormalities of mitoses, while those with a high proportion of these nucleoli showed polyploid and giant cells with various chromosome and spindle abnormalities. The abnormalities observed were: stickiness of chromosomes, nondisjunction of chromosomes, displacement of chromosomes, clumping, binucleate cells, polyploid cells, multinucleate cells, giant cells, and spindle abnormalities.

The aberrant chromosome and spindle behavior was attributed to stickiness and to increased rate of division, alterations that are caused by a quantitative change in the nucleic acid synthesis. The polymerization of thymonucleic acid is responsible for the coiling, the reduplication, and the visibility of chromosomes. The desoxyribose for the synthesis of thymonucleic acid may be obtained by reduction of the ribonucleic acid, which is produced under the influence of histone. An increase in the nucleic acid of tumors, as described by Caspersson and his co-workers, may be a fundamental cause of an increased rate of cell division and of malignancy. This quantitative change in nucleic acid metabolism, as indicated by abnormal chromosome behavior, may be an important differential criterion of a tumor cell.

In his discussion Koller suggests that in the first stages of neoplasia a disturbance in the heterochromatin-euchromatin balance or a slight increase in nucleic acid may cause a shortening of the resting stage between divisions that is not evident morphologically. An irregular segregation of the chromosomes at anaphase would produce further imbalance and increase in nucleic acid, giving more pronounced abnormal chromosome behavior and change in size and contents of nucleolus. An excess of nucleic acid makes the chromosomes sticky because they are coated with fluid nonpolymerized nucleic acid. In the next stage of the development of cancer there is nuclear division without cell division. In the final stage the nucleic acid content of the cells is further increased, abnormal

chromosome behavior and irregular division are frequent, and there are many giant polynucleate cells. These 3 stages, which were observed in the same and in different tumors, may be used as criteria of the degree of malignancy. It is known from genetic and cytologic evidence that the chromatic regions of the chromosomes, which are primarily concerned with nucleic acid synthesis, are more readily susceptible to mutation and structural change (43, 44, 92).

A chain of reactions exists in which the chromosome thread controls the polymerization of its thymonucleotide charge. This charge in turn controls the spiralization and the reproduction of the thread itself with its genes. The whole course of events can be altered by temperature and other cell conditions, as well as by the balance of heterochromatin and euchromatin and by the organization of the nucleolus. Koller suggests that the initial change in nucleic acid metabolism is brought about by a gene mutation occurring in the heterochromatic region responsible for the nucleic acid supply.

Biesele, Poyner, and Painter (13), using acetocarmine squash preparations, measured nuclear volumes and did chromosome counts on 10 mouse tumors, as well as on some normal adult and embryonic tissues of the mouse. They found that cancer nuclei fell into volume classes that were less distinct than those of nuclei of normal tissues. The Class I nuclei were small, diploid nuclei with chromosomes similar to those of normal diploid embryonic cells. Class II, the most frequent class in tumors, contained nuclei with chromosomes twice as large as normal and with approximately the diploid number. With these larger chromosomes, twice as many nucleolar organizers and twice as many plasmosomes were present. Class III nuclei, which were ideally twice as large as the Class II nuclei, contained tetraploid and diploid nuclei. The nuclei of Class IV contained octoploids with chromosomes twice the normal size, tetraploids with chromosomes 4 times normal size, and diploids with chromosomes 8 times normal size and strand number. Thirty-two nuclear organizers were present in the chromosomal complement. The other succeeding classes of nuclei similarly were presumably derived from the nuclei of the next lower class by endomitosis with or without division of the centromeres. The formation of diplochromosomes in carcinogenesis seemed irreversible. All cancer chromosomes except those in nuclei of Class I were larger than normal and had more strands. These observations agree with the general findings of other workers, which have been reviewed by Biesele (13), demonstrating that polytene chromosomes are widespread in the cancers of man and the lower animals, which are often characterized by greater nuclear volume, an increase in the number or size of nucleoli, and enlarged or doubled size of chromosomes. The increased basophilia of the cytoplasm of malignant cells is a result of higher concentration of ribonucleic acid associated with increased protein synthesis. Malignancy is paralleled in degree by frequency of endomitosis and concentration of nucleic acids.

Biesele's observations (7, 10, 12) on the chromosomes of an ovarian adenoma or adenocarcinoma of the goldfish, on 2 human mammary carcinomas, and on 2 rat neoplasms (hepatoma 31 and Walker carcinosarcoma 256) confirmed his work on mouse tumors. He concluded that the volume of the chromosomes in normal tissues does not vary in accordance with the cytoplasmic concentration of ribonucleic acid, nor with the relative development of heterochromatin and plasmosomes, but rather with the development of the euchromatin (9).

Biesele's suggestion (9) that there is a relationship between the volume of chromosomes, the euchromatin of the chromosomes, and the vitamin content of tissues is very interesting. However, since this hypothesis is based largely on numerous assumptions, for which direct evidence has not yet been obtained, we must await the accumulation of this information. Biesele's own measurements of chromosome volume do not correlate with his observations on the amount of ribonucleic acid in the cytoplasm of normal cells, so he assumes that the large size of the chromosomes in both normal and neoplastic cells is due to a disproportionate increase of euchromatin and not of heterochromatin. The euchromatin is thought to form more complex proteins than heterochromatin. He makes additional assumptions regarding the relationship between euchromatin and vitamin synthesis, on the basis of a correlation of his measurements on chromosome volume with the measurements of other workers who analyzed the vitamin B content of tissues and nuclei. Some of the measurements of these investigators were related to the weight of fresh tissue and others to the weight of dried tissue. One might question the significance of the correlation of measurements on chromosomes with other measurements that are not directly related to such criteria as amount per nucleus, per cell, or per unit volume of fresh tissue, since it is recognized that there are significant differences in the actual and relative size and water content of cells and nuclei in various normal and neoplastic tissues.

Biesele and Cowdry (11) found diplochromosomes, and even more greatly enlarged chromosomes, from the second day on to carcinoma production in the epidermal cells of mice painted with methylcholanthrene. Only normal chromosomes were present in the epidermis of control mice that were untreated or

painted with benzene. After comparing the chromosomes of normal and regenerating rat liver, Biesele (8) concluded that the appearance of polytene chromosomes in cancers is not a result solely of rapid growth in adult tissues. Polyploidia has also been observed by Thomas and Drew (124) in root tips grown in water containing dibenzanthracene. Additional evidence is needed to establish that the abnormalities of nucleic acid and of mitosis represent the fundamental process initiating neoplastic growth, and that they are not just another result of the aberrant functional physiology of neoplastic cells.

The one property common to all malignant cells is their capacity for unlimited, uncontrolled proliferation, which is evidenced cytologically by the abnormalities of cell division that have been mentioned. Whatever be the cause of neoplasia, the fact is clear that malignant cells are specifically altered cells and not merely normal cells excited to increased proliferation (Ludford, 84). It seems probable that this neoplastic alteration is transmitted through the nucleoproteins of the nucleus or cytoplasm.

Nucleoproteins in tumor inciting agents.—Certain agents that elicit the formation of neoplastic growths contain nucleic acids as one of their chief constituents. That the Shope papilloma virus contains desoxyribonucleic acid has already been mentioned (118). Kahler, Bryan, and Sipe (72) found that the substance in the milk of mice that incites mammary carcinomas contains a ribonucleic acid complex. Although the proof is not available, it is interesting to speculate on the possibility that these nucleic acids may exert their carcinogenic action by producing a disturbance of the normal nucleoproteins of cells.

SUMMARY AND CONCLUSIONS

Desoxyribonucleic acid, a most important constituent of the chromatin of cells, has a significant part in the transmission of hereditary characteristics by the genes, in mitosis, in nucleic acid synthesis and balance, and in the protein synthesis of the cell. The various studies on tumors by means of macrochemical analysis or visual inspection of Feulgen-stained material are not in agreement regarding disturbances of thymonucleic acid. Photometric histochemical observations, using the Feulgen reaction, have shown that some epidermoid carcinomas of mice and men, and leukemic blood cells from the human subject, contain increased amounts of thymonucleic acid. Cytochemical studies show that the cytoplasm of malignant cells contains increased amounts of ribonucleic acids, and suggest that the heterochromatic region of the chromatin plays a specific role in carcinogenesis. The observations in tumor tissues of stickiness, nondisjunction, displacement and clumping of chromosomes, of polyploid cells with increased number and volume of chromosomes, of more frequent mitoses, of enlarged nucleoli, and of multinucleate and giant cells represent cytologic evidence of abnormalities of nucleic acids in neoplastic cells. These findings are especially frequent in more anaplastic tumors.

Extracts of cells containing nucleic acids and their breakdown products have a growth-promoting effect on other cells. That desoxyribonucleic acid may induce a specific change in cells that is predictable and transmissible to subsequent generations has been shown in work with pneumococci. The Shope papilloma virus and the mammary tumor-inciting milk factor of mice, two agents that elicit tumors, contain desoxyribonucleic and ribonucleic acid, respectively. It is possible that nucleic acids may also induce transmissible changes in cells that become neoplastic.

Desoxyribonucleic acid is located in the chromatic regions of the chromosomes, which are susceptible to physical changes leading to mutation. It has been shown that similar wave lengths of ultraviolet light produce a breaking down of the polymerized form of sodium thymonucleate, mutations in chromosomes, and carcinoma of the skin. It is suggested that somatic mutations leading to neoplasia may be produced by alterations in the extremely complex macromolecule of thymonucleic acid. An initial slight modification in the complex polymerized thymonucleoprotein could, during a variable latent period, lead to a progressive and ultimately irreversible imbalance of nucleic acid—an imbalance characterized by the excessive amounts of ribonucleic acid found in rapidly proliferating cells. Quantitative or qualitative alterations in the nuclear and cytoplasmic nucleic acids and nucleoproteins—substances which seemingly directly or indirectly regulate many reproductive, synthetic and enzymatic activities of cells—could explain many of the properties of malignant cells. The presented evidence of a disturbance of nucleoproteins forms the basis for a relatively new concept of an intracellular cause of neoplasia, which will be established or disproved by subsequent investigation.

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ADDENDUM

Among the articles on nucleic acids published since this manuscript was prepared, three reviews are of especial interest. Greenstein has published a general review on nucleoproteins. Haddow discussed the possible relation of cytoplasmic structures containing nucleic acids to viruses and the malignant properties of cells, and Davidson and Waymouth reviewed the literature on nucleic acids and tissue growth.

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The Relative Thymonucleic Acid Content of Human Normal Epidermis, Hyperplastic Epidermis, and Epidermoid Carcinomas*†

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There is increasing evidence that neoplasia may be associated with a disturbance in the nucleoproteins and nucleic acid of cells (12). Previous investigations, carried out by means of a photometric histochemical method, have shown that some epidermoid carcinomas induced by painting the skin of mice with methylcholanthrene contain an increased amount of thymonucleic acid (10). The present study on epidermoid carcinomas of human skin was undertaken to expand the observations made on mice and to determine whether the amount of thymonucleic acid in epidermoid carcinomas was greater in the more anaplastic tumors.

MATERIALS AND METHODS

Specimens from epidermoid carcinomas of the skin were collected from patients at the Barnard Free Skin and Cancer Hospital by biopsy or operation, fixed in a mixture of equal parts of a saturated aqueous solution of mercuric chloride and 95 per cent ethyl alcohol, dehydrated in graded alchohols, cleared in xylol, and embedded in 56° paraffin. The specimens were embedded on different days, but care was taken to use a standard procedure.

Sections were cut on the same day from all the blocks and stained with hematoxylin and eosin. Two of the 13 specimens were considered unsatisfactory for measurement because of extensive infiltration with polymorphonuclear leukocytes and degeneration of tumor cells. The 11 specimens that were selected for measurements are listed in Table I. Measurements were made on 2 different blocks of specimens 2 and 4.

The tumor in specimen 1 was removed by cautery in September, 1942, but recurred in June, 1943. Similarly, specimen 6 was a recurrence of a tumor removed 3 months previously by cautery. Tumor 10 was a recurrent carcinoma, the primary tumor having been removed elsewhere with an electric needle 6 months previously. Specimens 3 and 5 had been treated with salves before removal. No other treatment was known to have been used on any of the tumors.

TABLE I: SPECIMENS OF SQUAMOUS CELL CARCINOMAS IN WHICH THYMONUCLEIC ACID WAS MEASURED

Tissue no.	Pathology specimen no.	Age and sex of patient	Location of tumor	Duration, months	Metastases	Other epidermoid carcinomas of skin
1	43-905	76 M	Rt. preauricular	34	0	0
2	43-952	64 M	Rt. hand	36	0	0
3	43-958	53 M	Cheek	36	0	1
4	43-968	62 M	Left leg	24	+	1
5	43-985	54 M	Rt. ear	24	0	0
6	43-1094	71 M	Left wrist	7	0	0
7	43-1128	72 M	Rt. hand	12	0	0
8	43-1132	75 M	Left hand	3	0	0
9	43-1087	75 M	Lower lip	84	0	0
10	44-37	75 M	Left hand	24	0	0
11	44-156	78 M	Rt. hand	30	0	0

With photometric apparatus consisting of a stable light source, light filters, a microscope, a photocell, and amplification and recording apparatus (10) the relative amounts of pigment in tissues can be determined. When a stain is employed that is specific for one component of the tissues, the relative amounts of

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that substance can be measured. Thymonucleic, or desoxyribonucleic, acid is stained with relative specificity by the Feulgen reaction (13). Under the controlled conditions of these experiments, the assumption seems justified that the amount of stain in the tissues is proportional to the amount of thymonucleic acid in the cells.

In a comparable manner to that described in previous experiments (10, 11) the sections of tissue were mounted and stained simultaneously under carefully controlled conditions. The 13 different sections were grouped together on 5 different slides. Two sets of slides of adjacent serial sections were stained by the Feulgen reaction and a third set was prepared as unstained control. The staining procedures and methods of obtaining the measurements with the photometric apparatus have been described (10). The diaphragm in the ocular of the microscope limited the stage field to an area measuring 32 by 42 microns. Since in the areas measured the light passed through a uniform thickness of tissue, the results of the absorption per area may be correctly considered as the absorption of a definite volume of tissue.

Whenever possible normal epidermis, hyperplastic epidermis, and carcinoma were measured in sequence on the same section on the same day. In measuring the second series of slides, this sequence of measurements was reversed. On some sections it was possible to measure only carcinoma, or carcinoma and either normal or hyperplastic epidermis. Sketches were prepared of each section and an attempt was made to measure the same area on each of the 2 adjacent serial sections. A total of 50 areas was measured on each of the duplicate sections, as well as on the unstained control sections. During the measurements the number of cells in each area was recorded so that the amount of absorption per cell could be determined. Areas showing necrosis, keratinization, pigmentation, vacuolation, or infiltration of inflammatory cells were excluded from the measurements.

The mean measurements of the absorption per area for the normal epidermis, hyperplastic epidermis, and carcinoma were compared with each other to determine their statistical significance. This was done by determining the ratio, $\frac{D}{\sigma_D}$, of the difference between the means of the measurements and the standard error of the difference between the two means. From this ratio the probability, P, of obtaining a difference as large or larger by accident was determined by reference to statistical tables.

RESULTS

The results of each set of 50 measurements of the normal epidermis, hyperplastic epidermis, and epidermoid carcinomas are shown in Table II. The first

TABLE II: RELATIVE THYMONUCLEIC ACID CONTENT PER AREA AND PER CELL OF NORMAL EPIDERMIS, HYPERPLASTIC EPIDERMIS, AND EPIDERMOID CARCINOMAS

Tissue no. and type	Per cent absorption per area	Coefficient of variation, %	No. of cells per area	Percentage aborption per cell
1—Normal—1	12.8	22.4	12.6	1.01
" 2	12.4	25.5	11.6	1.07
Hyperplastic—1	11.8	36.8	12.9	.91
" 2	9.9	46.7	10.8	.91
Carcinoma—1	16.3	16.7	13.2	1.23
" 2	16.9	29.6	11.3	1.50
2a—Hyperplastic—1	13.0	35.0	13.2	.98
" 2	9.1	28.6	8.9	1.02
Carcinoma—1	18.3	21.7	17.1	1.11
" 2	16.1	23.4	15.8	1.02
2b—Normal—1	14.0	30.2	16.3	.86
" 2	13.9	27.9	15.8	.87
Hyperplastic—1	12.1	39.9	12.5	.97
" 2	10.0	39.4	9.4	1.06
Carcinoma—1	16.1	22.5	15.6	1.03
" 2	15.9	24.2	13.7	1.16
3—Normal—1	15.6	14.9	11.4	1.37
" 2	17.5	15.5	13.4	1.31
Hyperplastic—1	10.6	31.7	10.6	1.00
" 2	11.8	26.5	10.5	1.12
Carcinoma—1	15.7	22.5	14.0	1.12
" 2	17.5	8.7	16.4	1.07
4a—Hyperplastic—1	13.7	38.5	13.3	1.03
" 2	12.9	32.6	11.6	1.11
Carcinoma—1	17.8	14.8	15.8	1.12
" 2	18.9	26.8	14.7	1.28
4b—Hyperplastic—1	12.1	27.9	12.9	.93
" 2	10.3	24.8	10.8	.95
Carcinoma—1	15.0	33.6	13.7	1.09
" 2	14.7	21.5	13.2	1.07
5—Normal—1	14.9	17.2	13.2	1.13
" 2	14.9	29.7	11.0	1.36
Carcinoma—1	20.5	16.0	17.2	1.19
" 2	20.8	9.2	17.4	1.19
6—Carcinoma—1	13.6	22.4	13.8	.98
" 2	12.8	21.9	12.1	1.06
7—Normal—1	13.0	33.6	14.0	.93
" 2	14.8	38.7	13.2	1.12
Hyperplastic—1	8.4	48.5	8.2	1.02
" 2	9.7	45.7	8.7	1.12
Carcinoma—1	13.2	27.6	10.7	1.23
" 2	13.5	20.9	10.7	1.26
8—Normal—1	14.2	39.4	13.5	1.05
" 2	15.3	31.6	13.1	1.17
Hyperplastic—1	12.3	25.3	11.9	1.03
" 2	12.1	33.7	9.1	1.30
Carcinoma—1	15.8	21.5	13.9	1.13
" 2	16.5	19.1	14.3	1.15
9— " 1	14.2	33.1	12.9	1.10
" 2	12.8	16.1	11.6	1.10
10—Hyperplastic—1	12.5	41.8	12.7	.98
" 2	10.4	41.5	10.3	.97
Carcinoma—1	14.8	19.8	13.8	1.07
" 2	13.9	17.3	14.1	.98
11—Hyperplastic—1	10.0	37.6	9.0	1.11
" 2	11.9	35.1	10.7	1.11
Carcinoma—1	17.7	23.4	11.1	1.60
" 2	18.4	19.3	10.6	1.70

and second section of each block are indicated by the numbers 1 and 2. The mean absorption per area was divided by the mean number of cells per area to give a figure for the mean absorption per cell. The figures for the 2 sets of measurements of 50 areas on the same type of tissue from 1 biopsy usually showed close agreement. The coefficient of variation, which is an indication of the variability of absorption in different areas of any one type of tissue, was highest for the hyperplastic epidermis. The mean coefficients of variation for the measurements of the absorption per area for 12 sections of normal epidermis, for 20 sections of hyperplastic epidermis, and for 26 sections of carcinomas were 27.2, 35.9, and 21.3 per cent respectively.

between these means the value of the ratios was taken as the figures for computing one mean and 1 was used as the figure for the other mean. For determining the standard error of the mean for these small groups N minus 1 was employed for the number of observations.

For the normal epidermis, hyperplastic epidermis, and carcinomas, the respective mean percentages of absorption per area were 14.4, 11.2, and 16.1. The corresponding mean absorptions per cell were 1.10, 1.03, and 1.17 per cent. There was an average of 13.3, 10.9, and 13.8 cells per area in the normal, hyperplastic, and neoplastic epithelium.

All the means of 50 measurements for normal epidermis, hyperplastic epidermis, and carcinoma were

TABLE III: THE CELLS PER AREA AND RELATIVE THYMONUCLEIC ACID CONTENT PER AREA AND PER CELL EXPRESSED AS RATIOS OF MEAN ABSORPTION OF CARCINOMA TO NORMAL EPIDERMIS, $\frac{C}{N}$; HYPERPLASTIC EPIDERMIS TO NORMAL EPIDERMIS, $\frac{H}{N}$; AND CARCINOMA TO HYPERPLASTIC EPIDERMIS, $\frac{C}{H}$

Tissue no.	Cells per area			Absorption per area			Absorption per cell			Grade of tumor
	$\frac{C}{N}$	$\frac{H}{N}$	$\frac{C}{H}$	$\frac{C}{N}$	$\frac{H}{N}$	$\frac{C}{H}$	$\frac{C}{N}$	$\frac{H}{N}$	$\frac{C}{H}$	
1	1.01	.98	1.03	1.32	.86	1.54	1.31	.88	1.49	III
2a			1.49			1.60			1.07	I
2b	.91	.69	1.33	1.14	.79	1.46	1.26	1.16	1.08	II
3	1.23	.86	1.43	1.00	.68	1.48	.82	.79	1.04	II
4a			1.23			1.38			1.12	III
4b			1.14			1.30			1.14	III
5	1.43			1.38			.96			III
7	.79	.62	1.27	.96	.65	1.48	1.22	1.05	1.16	II
8	.86	.87	.98	1.09	.83	1.32	1.03	1.04	.99	I
10			1.22			1.28			1.05	III
11			1.06			1.77			1.48	I
Mean ratio	1.04	.80	1.22	1.15	.76	1.46	1.10	.98	1.16	
Mean P	.3336	.0003	.0000	.0102	.2236	.0000	.0869	.3707	.0012	

The mean absorption per area of the unstained control sections was 0.5 per cent. All the series of measurements were completed within a period of 30 days. In sections measured at different times there was no evidence of any consistent difference due to such possible factors as variation in the apparatus or fading of the stain.

To compare the number of cells per area and the amounts of thymonucleic acid per area and per cell in normal epidermis, hyperplastic epidermis, and carcinomas in the same biopsy specimen, ratios were determined as shown in Table III. Here the mean of the measurements on both corresponding sections was used in the calculations. The means for each group of ratios as well as the probability, P, of obtaining such differences by chance were computed. Values of P greater than 0.01, which indicate more than one chance in a hundred of getting as great a difference by chance, are not significant differences. In determining the standard error of the difference considered as separate groups, and the coefficient of variation of each group was determined. For the normal epidermis, hyperplastic epidermis, and carcinomas, the coefficients of variation of the percentages of absorption per area were 9.3, 12.6, and 17.9 per cent respectively; of the percentages of absorption per cell 15.4, 8.9, and 17.9; and of the number of nuclei per area 11.5, 14.8, and 14.7. Thus the mean absorption per area and per cell of different carcinomas varied more than the means of normal or hyperplastic epidermis.

As an indication of the statistical significance of the differences between the mean values for absorption per area and per cell, the figures for the probability, P, are shown in Table IV. A minus sign preceding a figure for P indicates that the figure applies to the chances for a negative instead of a positive difference between the two means. The mean of the values for $\frac{D}{\sigma_D}$ for the normal and hyperplastic epidermis was

4.5, for hyperplastic epidermis and carcinoma 5.8, and for normal epidermis and carcinoma 3.6. All these mean ratios gave significant values for P of 0.0002 or less.

The degree of anaplasia of the tumors was determined for the particular part of the carcinoma measured. The grade of each tumor is shown in Table III. There was no significant correlation between the grade of the tumor and the amount of thymonucleic acid per nucleus.

Of more importance than the amounts of thymonucleic acid per unit volume of tissue were the relative amounts of thymonucleic acid per cell. The nuclei of most epidermoid carcinomas contained more thymonucleic acid than those of hyperplastic epithelium and more than the majority of specimens of normal epidermis. As compared with the nuclei of normal cells, the nuclei of the hyperplastic epithelium contained increased amounts of thymonucleic acid in some instances and decreased amounts in other biopsy specimens. These variations in the mean amount of thymonucleic acid in the nuclei of the cancer cells, as compared with that found in the nuclei of normal or hyperplastic epithelium, are additional evidence of a disturbance of nucleic acids in cancer.

Table IV: Statistical Significance of Differences between Absorptions Per Area and Per Cell of Normal Epidermis, *N*; Hyperplastic Epidermis, *H*; and Epidermoid Carcinomas, *C*

	Absorption per area			Absorption per cell		
Tissue no.	P of *C-N*	P of *C-H*	P of *N-H*	P of *C-N*	P of *C-H*	P of *N-H*
1	0.0000	0.0000	0.0150	0.0000	0.0000	0.0000
2a		0.0000			0.4692	
2b	0.0071	0.0000	0.0003	0.0000	0.0968	—0.0002
3	0.4641	0.0000	0.0000	—0.0119	—0.1251	0.0003
4a		0.0000			0.0000	
4b		0.0003			0.0869	
5	0.0000			0.0485		
7	0.1038	0.0000	0.0000	0.0000	0.0000	—0.0823
8	0.0655	0.0000	0.0027	0.0951	—0.1357	—0.0174
10		0.0000			0.0465	
11		0.0000			0.0000	

In an attempt to evaluate the relationship of nuclear size to the content of thymonucleic acid per nucleus, measurements were made of relative nuclear volume. This was done by projecting the areas of the sections on which the photometric measurements had been made and tracing the nuclear outlines on kodaloid (Eastman Kodak Co.). The volumes were calculated by 2 different methods. One method employed the formula for an ellipsoid, $V = 4/3\pi a.b.c$, in which a, b, and c are the mean semi-axes of the nuclei. For these measurements a was the semi-major axis, b the semi-minor axis of the tracing, and the third semi-axis, c, was assumed to be the same as b, since it is difficult to measure the thickness of nuclei in sections. Such a formula would approximate the correct nuclear volume only when all nuclei were sectioned on the plane of the major axis—a condition that would not exist in sections of tumors. The second formula was derived by G. H. Scott and V. Rojansky, both formerly of Washington University. The derivation of this formula has not been published, although it has been used by several investigators, including Cowdry and Paletta (4).

Because of the wide discrepancy in the results from the application of these 2 formulas, paraffin models of 100 nuclei of the carcinoma in biopsy number 7 were prepared from outline projection tracings and cast in a block of plaster of paris. After cutting the block into slabs, corresponding to sections of the tumor, the maximum nuclear outlines were traced on kodaloid and the nuclear volume was determined by both methods. The true volume of the paraffin models of the nuclei was determined by comparison of their mean weight with the weight of a known volume of the paraffin. The first formula gave volumetric measurements that were 42 per cent below the true value. The measurements from the second formula were 45 per cent higher than the correct volume. Therefore, the average of both methods was taken as the corrected volume for these nuclear comparisons.

In biopsies 1, 2, 7, and 11 a total of 250 nuclei was measured in the different parts of each section. As compared with normal nuclei, the mean volume of the nuclei of hyperplastic epithelium and carcinoma cells was increased 40 and 115 per cent respectively. The mean volume of the nuclei of the carcinomas was 85 per cent greater than that of the hyperplastic epidermis. The correlation coefficient of the mean per cent absorption per cell and mean nuclear volume was 0.6. By dividing the mean per cent absorption per nucleus by the mean nuclear volume, one obtains a relative figure for the mean absorption per unit volume of nucleus. These data show that, as compared with normal epithelium, there was 29 and 46 per cent less absorption per unit volume of nucleus

in hyperplastic epithelium and carcinomas. This decreased absorption could be caused by an artifact, because in large nuclei more than enough stain may be present to produce a focal complete absorption of light in the photometric apparatus. That this is by no means the complete explanation is evident from the fact that the ratios of nuclear size and absorption per unit volume of tissue do not show an equal or proportional decrease as the size of the nuclei increases. Some of the decrease in mean absorption, therefore, is caused by a decreased concentration of thymonucleic acid per unit volume of nucleus in the larger cells of some specimens of hyperplastic epithelium and carcinomas.

DISCUSSION

It is generally agreed that under properly controlled conditions the Feulgen reaction is a relatively specific stain for desoxyribonucleic acid (2, 6, 7, 10, 13). The assertions of Stedman and Stedman (8, 9) and Choudhuri (3) that it is chromosomin, and not thymonucleic acid, that stains by the Feulgen reaction have been answered by Barber and Callan (1). The developed nuclear stain has the same absorption curve as chromatin stained by the Feulgen reagent (Stowell and Albers, 14), and the staining procedure described by Choudhuri probably represents an absorption of pigment by the chromatin. The present work is based on the assumption that the relative amount of pigment in the sections of tissue is proportional to the amount of thymonucleic acid present.

The mean amount of thymonucleic acid per area decreased 24 per cent in hyperplastic epithelium as compared with normal cells. This change, which was accompanied by a decrease of 20 per cent in the number of cells per unit volume of tissue, is explained by the increase in the size of the individual cells. The mean amount of thymonucleic acid per cell was increased in some specimens of hyperplastic epidermis and decreased in others. These results on human epidermis agree with the results in mice (10).

As compared with the hyperplastic epithelium, the tissues of epidermoid carcinoma contained an average of 46 per cent more thymonucleic acid per unit volume. The mean number of cells in the carcinomas was increased 22 per cent, which meant that the carcinoma cells were smaller than the cells of the hyperplastic epithelium. Since the mean size of the carcinoma nuclei was larger than that of the hyperplastic epithelium, the carcinoma cells contained relatively less cytoplasm. The malignant cells had an average of 16 per cent more thymonucleic acid per cell than the cells of hyperplastic epidermis.

In comparing the carcinoma cells with the normal epidermis, there was found an average of 15 per cent more thymonucleic acid per area, 4 per cent more cells per area, and 10 per cent more absorption per cell. The values for these last two means were not statistically significant, because the differences were significantly increased in some instances and slightly decreased in others. It is probably of more significance to compare the thymonucleic acid content of normal epidermis, hyperplastic epidermis, and carcinomas on the same biopsy specimen than on biopsy material from different patients. This was conveniently done by means of the ratios shown in Table III. For the 2 sections from different blocks of the same tumor (2a and b, and 4a and b) the results agreed more closely than for most of the other sections. As an example of the obvious differences in the thymonucleic acid content of hyperplastic epithelium and epidermoid carcinoma, 2 areas from biopsy number 11 are shown in Figs. 1 and 2. The photographs were taken from one of the areas measured. The same Wratten filter was used in the photography as was employed to give complementary light in the photometric apparatus. Essentially all the light absorption is produced by the stained thymonucleic acid within the nucleus. As compared with the hyperplastic epithelium, the neoplastic tissue in this specimen contained an average of 66 and 48 per cent more desoxyribonucleic acid per unit volume and per cell respectively.

Different specimens show considerable variation in the mean content of thymonucleic acid in their normal and neoplastic tissues. The explanation of why the thymonucleic acid content per cell should be increased in some carcinomas and decreased in others is not evident. However, many of the properties of cancer cells show great variation between individual tumors. For example, Cowdry and Paletta (4, 5) found considerable variation in human epidermoid carcinomas in the nuclear size and nuclear viscosity. For the relatively small number of specimens measured there is no significant correlation between the degree of anaplasia of the tumor and the mean amount of thymonucleic acid per cell. The changes are not entirely explained on the basis of larger nuclei containing more desoxyribonucleic acid. In fact the measurements suggest that some carcinomas and hyperplastic epithelium have a decreased content of thymonucleic acid per unit volume of nuclear mass.

In considering the observations on human epidermis and epidermoid tumors, the material reviewed elsewhere on thymonucleic acid (12) will not be repeated. In general, the observations on squamous cell tumors in mice have been confirmed by these studies of biopsies from patients. Why the thymonucleic acid content per cell, which is relatively constant for normal and hyperplastic epidermis, is increased in many carcino-

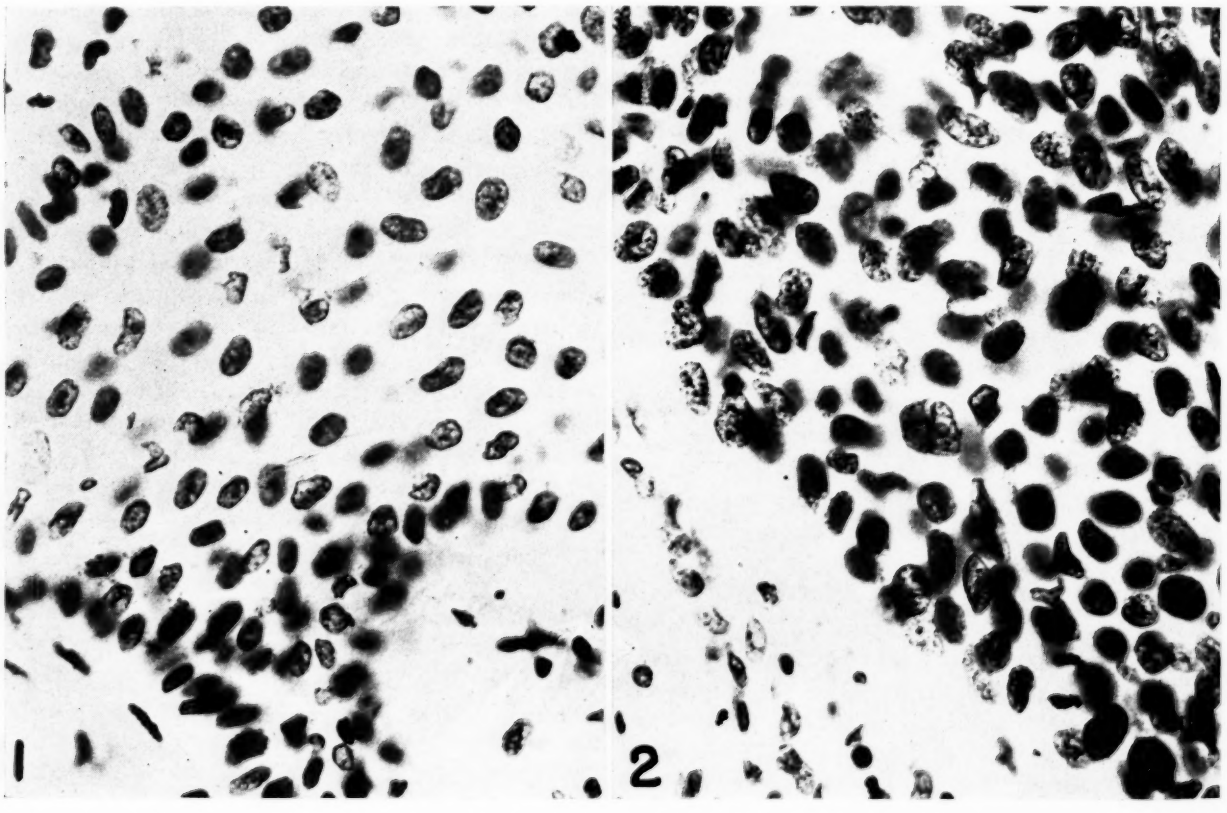
mas and decreased in a few others has not yet been determined. Such fluctuations are, however, additional evidence of the disturbance in nucleoproteins that occurs in many tumors. The necessary evidence is certainly not yet available to suggest that all tumors are fundamentally caused or accompanied by a disturbance of the normal balance of nucleic acids.

SUMMARY AND CONCLUSIONS

With the Feulgen technic and a microphotometric apparatus, measurements have been made of the relative amounts of thymonucleic acid in normal epidermis, hyperplastic epidermis, and in epidermoid carcinomas of the skin. The measurements were made under carefully controlled conditions on adjacent areas of sections of 13 biopsy specimens from the tumors of 11 patients.

The results were similar to the previous observations on experimental carcinogenesis in the skin of mice. Hyperplastic epidermis, in which the cells were large, contained less thymonucleic acid per unit volume of tissue than normal epidermis or carcinomas. The carcinomas contained more thymonucleic acid per unit volume of tissue than the normal epidermis.

As compared with normal and hyperplastic epithelium, the nuclei of some carcinomas contained increased amounts of thymonucleic acid per cell that were statistically significant, and in no instance was it significantly decreased. These variations in the mean amount of thymonucleic acid in the cells of epidermoid carcinoma show that in some types of neoplasia there is a disturbance of the nucleic acids. No significant correlation was observed between the mean thymonucleic acid content per cell and the degree of anaplasia of the tumors. Additional work is necessary to determine whether this disturbance of nucleoproteins is a cause or a result of the malignant transformation.



FIGS. 1 and 2.—Areas from biopsy specimen 11, showing hyperplastic epidermis (Fig. 1) with a low content of thymonucleic acid and epidermoid carcinoma (Fig. 2) with a high content of thymonucleic acid per area and per cell. Section 10 microns thick, stained by Feulgen reaction. Mag. × 460.

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Respiratory Behavior of Bacteria-Free Crown-Gall Tissues

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INTRODUCTION

The respiratory processes of malignant tissues of animals and human beings have long been considered to differ in important respects from those of non-malignant tissues of the same creatures. This difference expresses itself in several ways, especially in a low R.Q. (0.8 or less) and a decided anaerobic tendency to accumulate acid products, lactic acid in particular. It seems possible, indeed, if we may draw an analogy from plant materials (6, 9), that the characteristic independence from normal morphogenetic restraint shown by tumor tissues may be essentially an expression of independence from the formative influences of oxygen gradients. These evidences of a high degree of anaerobiosis are considered typical of malignant growths, and their possible use in the diagnosis of malignancy has been suggested by some (2, Footnote, p. 242).

Routine observations on *in vitro* cultures of tissues from cancer-like, bacteria-free, crown-gall tissues of sunflower (12) disclosed 3 traits that suggest the existence of a similar picture in plant tumors. In the first place, old cultures have a distinctly yeasty or alcoholic odor. Since this odor, in yeasts, is an accompaniment of an anaerobic type of respiration, it may be interpreted in plant tumor materials as suggesting the accumulation of incompletely respired materials even when oxygen for complete respiration is available to the tissues. Since these cultures are bacteriologically sterile this accumulation must result from respiratory processes in the tumor tissues themselves. In the second place, such cultures tend to acidify the subjacent nutrient (unpublished data), indicating again the accumulation of incompletely respired, in this case acid, products. There seems to be no general agreement as regards the behavior of normal plant tissues in this respect. Robbins and Maneval (5) found that excised corn root tips tended to raise the pH of the nutrient, while White (7) reported a lowering of pH by wheat root tips. In the third place, such cultures, when immersed in a liquid nutrient, do not undergo the formative changes evinced by similarly treated cultures of *Nicotiana*-hybrid tissues, which changes have been attributed to effects of an altered oxygen gradient [1] (9). These observations, together with the obvious possibility of an analogy between these plant tumor cells and those of animal tumors, led to a more careful examination of the respiratory processes involved. The work reported here is the result of preliminary studies on this problem.

MATERIALS AND METHODS

Interest centered particularly around the behavior of tissues of the bacteria-free crown-gall tumors of sunflower, which have been shown to possess in high degree the properties of malignancy; transplantability, and the quantitatively and qualitatively unrestrained growth commonly associated with animal and human cancers (12). The respiratory processes of 3 types of bacteria-free tumor tissues of sunflower were studied: (a) secondary tumors arising as a direct result of the inoculation of young sunflower plants with crown-gall bacteria (1); (b) tissue cultures isolated from secondary tumors and maintained *in vitro* through repeated (40 or more) passages (12); and (c) tumors arising as a result of implantation of fragments of tissue cultures under the bark of otherwise healthy plants (12). The respiration of these 3 types of bacteria-free crown-gall tumors were compared with a second type of bacteria-free plant tumor, of abiotic origin; that arising in consequence of hereditary defect in tissues of plants resulting from the cross *Nicotiana langsdorffii* × *N. glauca* (4) represented by: (a) tissue cultures obtained from the procambial region of such plants and maintained *in vitro* for many (100 or more) passages (8); and (b) tumors resulting from implantation of such tissue cultures under the bark of otherwise healthy plants of *Nicotiana glauca* (11). All these were further compared with tissues of bacteria-containing crown-gall tumors of sunflower, with healthy tissues of sunflower represented by internodal segments taken at a distance of 3 to 4 cm.

[1] Skoog, however, (unpublished, personal communication) gives a quite different explanation of this failure to differentiate under reduced oxygen supply.

from transplant galls, and with stem growing points and young inflorescences from healthy plants. Records were thus obtained from 3 types of healthy tissue, from bacteria-containing tissue, and from 5 types of bacteria-free tumor tissue, including material of 2 unrelated host plants.

Respiration of these tissues was studied in a standard Barcroft-Warburg apparatus. The vessels used were of about 7.0 ml. capacity and of the standard type, with center well for caustic paper and a single side arm for acid. For most experiments 0.5 ml. of White's nutrient (10) containing 2 per cent sucrose was used to bathe the tissues; this was just enough to keep the tissues thoroughly moist, but not enough to cover them. A like amount of 20 per cent KOH was employed in the center well with a filter-paper roll. Tissues to give wet weights of between 30 and 300 mgm. were torn into fragments small enough to enter the flasks comfortably, tearing rather than cutting being considered preferable as introducing a lesser degree of trauma. All tissues were dried after respiratory test to constant weight at 120° C. and all calculations were reduced to cu. mm. of gas absorbed or released per mgm. dry weight of tissue. The manometers were shaken at an arbitrarily chosen speed of 120 r.p.m. with a 2.5 cm. stroke, though in view of the small volume of fluid used shaking is, of course, probably of little significance. The bath was kept at $25° \pm 0.1°$ C. Most series of experiments were run in sevens; 6 flasks containing tissue, the seventh serving as thermobarometer.

Theoretically, the use of dry weights in dealing with plant tissues, with their relatively high and often widely differing content of inert storage carbohydrate, introduces an unknown and possibly important error into the calculations (see also discussion by Burk, 3, p. 207-208). Protein nitrogen should give a much better basis for comparison of the respiratory activity of plant tissues. Actually, however, the water content of the tissues studied here was relatively high and fairly constant. The highest individual dry weight recorded was 19 per cent, in a flower bud, which class had an average dry weight of 11.6 per cent and a range from 9.7 to 19.0. Bacterial tumors, and bacteria-free tumors arising from grafting of tissue cultures, had essentially identical percentage dry weights—11.6 (8.8 to 15.0) per cent, and 11.8 (8.4 to 17.1) per cent respectively. Healthy stem growing points were slightly lower—10.5 (8.0 to 15.0) per cent, and healthy internodal tissue was still lower—8.1 (3.6 to 12.9) per cent. Tissue cultures from *Nicotiana* had slightly higher dry weights than these last—8.7 (5.4 to 15.8) per cent, while *Helianthus* tumor tissue cultures were again lower—7.3 (3.3 to 12.9) per cent. Yet the widest range of averages, from 11.8 per cent dry weight for *Helianthus* graft tumors to 7.3 per cent for *Helianthus* tumor tissue cultures, represents a difference of only 40 per cent, based on the higher figure. Some of this dry weight is certainly inert in all tissues studied, so that all Q_{O_2}'s [2] must be considered minimal values. And much of the variation between Q_{O_2} values for different samples may be due to differences in content of respiring protoplasm. The over-all picture, however, seems unlikely to be materially altered by this factor. As long as we realize the existence of this uncertainty and weigh our interpretations accordingly, dry weight can be employed as a useful though not fully satisfactory basis of reckoning.

Since many readers of this journal may be unfamiliar with the metabolic characteristics of plant materials, and since a discussion of respiration in plants involves certain factors not present in animal tissues, it may be desirable to review these matters briefly.

Respiration, as defined in plant physiology, is *any vital process that frees energy for the use of the organism*. This is to be clearly distinguished from the mechanical exchange of gases, which in higher animals is the most obvious accompaniment of this process. The two are sometimes distinguished as *energesis* and *breathing*. In higher organisms, both plant and animal, the commonest type of respiration is the oxidative catabolism of carbohydrate, but it should be kept in mind that there are processes like lactic fermentation that release energy by means of a drop in energy level of the internal system ($C_6H_{12}O_6 \rightarrow 2C_2H_4OH \cdot COOH + 18$ Cal.). These are also respirations under the definition given above, although no "oxidation" in the usual sense takes place.

When the oxygen involved in carbohydrate catabolism is in the form of the free gas, the process is known as *aerobic respiration*. When free oxygen is not involved, the oxygen coming from internal sources, the process is called *anaerobic respiration*. Although anaerobic respiration commonly occurs where free oxygen is not available, it should be remembered that it may also occur in the presence of free oxygen, the crucial question being not what gases are available, but rather what gases are utilized. Yeasts, for example,

[2] These symbols, Q_{O_2}, Q_{CO_2}, Q_{N_2}, etc., refer to the amounts of the designated substances involved in the processes under discussion. Thus the Q_{O_2} is the amount of oxygen, measured in cu. mm., absorbed by a mgm. dry weight of tissue in an hour. The superscripts, $Q_{O_2}^{Air}$, $Q_{O_2}^{O_2}$, $Q_{CO_2}^{N_2}$, etc., indicate the conditions under which a given test is made, whether in air, oxygen, nitrogen, helium, etc. R.Q. is the ratio of oxygen absorbed to carbon dioxide evolved, and is calculated as $\frac{Q_{CO_2}}{Q_{O_2}}$. These expressions are familiar to students of respiration but may be unfamiliar to the general reader.

regularly respire anaerobically even in high oxygen atmospheres. Palladin, indeed, believed that all CO_2 produced in oxygen respiration is of anaerobic origin, the oxidative process going on at the expense of the pigment and not of the carbohydrate.

In aerobic respiration there is a patent absorption of oxygen, which can be measured manometrically, and is expressed as Q_{O_2}.[2] This is further elaborated as $Q_{O_2}^{Air}$ or $Q_{O_2}^{O_2}$ to distinguish the two common types of gaseous media in which it is determined.

Complete oxidative catabolism of carbohydrate gives rise to CO_2 and H_2O, so that a second significant respiratory criterion is the Q_{CO_2}. This can be measured continuously by electrometric methods, or intermittently by absorption in caustic in the Pettenkoffer apparatus. In the Barcroft-Warburg apparatus it cannot be measured continuously in air or oxygen, but only as a final over-all reading by absorption in caustic, as in the Dixon-Keilin method. It *can* be measured continuously in nitrogen, since here the total gas pressure change is due to evolution of CO_2. We therefore can record the $Q_{CO_2}^{N_2}$ as a direct measure of anaerobic respiration.

When oxidative catabolism goes to completion the total reaction may be expressed by the equation $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 674$ Cal. In this case the amounts of CO_2 and O_2 are equal and the process may be characterized by the quotient $\frac{CO_2}{O_2} = 1$. This is called the R.Q. When the Barcroft-Warburg apparatus is employed in its simplest form, without caustic, the readings obviously represent the algebraic sum of the exchange of these two gases, $Q_{CO_2+O_2} = \frac{Q_{CO_2}}{Q_{O_2}} = \text{R.Q.}$ If the R.Q. is 1 (complete and unmodified respiration) there will be no change in volume of the gaseous system, and the readings will be consistently zero.[3] Any increase in volume of the system (positive readings) would represent an excess of CO_2 over O_2, hence an anaerobic respiration; any decrease in volume (negative readings) would represent an excess of O_2 over CO_2, as occurs in the oxidation of fat. Since complete anaerobiosis would give $\text{R.Q.} = \frac{nCO_2}{oO_2} = nCO_2$, and since Q_{CO_2} can be measured directly in nitrogen, the degree with which the directly read apparent R.Q. departs from the zero line in a positive direction (Fig. 2, left hand column) and approaches the $Q_{CO_2}^{N_2}$ (Fig. 2, second column) is a measure of the relative completeness of anaerobiosis in the presence of oxygen; that is, of the degree to which anaerobiosis is obligate.

Anaerobic respiration in animal tissues commonly takes the form of incomplete degradation of carbohydrate to lactic acid, according to the formula $C_6H_{12}O_6 \rightarrow 2C_2H_4OH \cdot COOH + 18$ Cal. Here there is no gas exchange, but only a change in energy level within the system. Such a reaction is commonly called "glycolysis."[4] In animal tissue studies glycolysis has come to be used as synonymous with lactic acid fermentation; it should be remembered, however, that this is only a special case. Any degradation of carbohydrate involving hydrolysis, carboxylation, or hydroxylation, whether it be to lactic acid, butyric acid, or alcohol, is essentially a glycolysis in the same sense. Since anaerobic respiration studies with animal tissues are generally carried out in media containing bicarbonate, the lactic acid can be calculated from the CO_2 displaced from the bicarbonate, and is generally expressed as Q_G^{Air}, $Q_G^{O_2}$ or $Q_G^{N_2}$, the G representing glycolysis. Measurement of anaerobic respiration in plants encounters two differences; first, that lactic acid is not the typical end product in plant tissues, and second, that plant tissue nutrients generally do not contain carbonate or bicarbonate. The characteristic anaerobic degradation (fermentation) in plants is to alcohol, according to the formula $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 28$ Cal. Here no oxygen is absorbed, but CO_2 *is* released and can be determined directly by absorption in caustic. One can thus calculate a figure for Q_G^{Air} (or $Q_G^{O_2}$) by subtracting the observed Q_{O_2} from the observed $Q_{CO_2+O_2}$. This difference is of course synonymous with the Q_{CO_2}—it is, in fact, a real Q_{CO_2} even in

[3] Although commonly so stated this is not strictly true, since the solubilities of O_2 and CO_2 differ. This difference is, in fact, the basis for the so-called "Direct Method of Warburg." The difference in solubility makes the slope of the curve dependent not only on the volumes of gas absorbed or evolved but also on the total flask volume, and the volume of fluid present as well. The formula is

$$\frac{\Delta V}{\Delta t} = \frac{X_{O_2}[K_{CO_2} - \text{R.Q.} \cdot K_{O_2}]}{K_{O_2} \cdot K_{CO_2}}$$

If the volume of fluid used is large in proportion to the amount of gases present the error may be important. In these experiments, however, with a fluid volume of 0.5 ml. in a 7 ml. flask the error is small. A typical example (flask No. 1) gives

$$\frac{\Delta V}{\Delta t} = \frac{X_{O_2}[1.15 - (1 \times 1.09)]}{1.09 \times 1.15} = \frac{X_{O_2}(0.06)}{1.25} = 0.05X_{O_2}$$

in which case the error is 5 per cent of the volume of oxygen absorbed.

[4] The dictionary (Webster's, Stedman's) defines glycolysis as "hydrolytic decomposition of sugar." Strictly speaking, lactic or alcoholic fermentations, which involve formation of carboxyl or hydroxyl groups without addition of water (hydrolysis), are not glycolyses according to this definition. The use of the term to apply to lactic fermentation has, however, become so universal in animal respiration studies that it must be accepted. There seems, nevertheless, to be no justification for making it synonymous with *lactic* fermentation alone, as is so often done by animal physiologists.

the case of alcoholic fermentation, in contradistinction to the apparent Q_{CO_2} (really $Q_{lactate}$) observed in animal tissues. Only when its value is greater than the Q_{O_2}, however, does it signify a glycolysis.

Some authors designate these various quotients as positive or negative. These signs are merely the expression of change in volume of the closed Barcroft-Warburg system. The Q_{O_2} is thus characteristically negative, the Q_{CO_2} positive. One might, however, reason with equal logic that the Q_{O_2} represents a gain to the tissue, and should therefore be designated with a positive sign! Moreover, the use of signs leads to the anomalous result that the respiratory quotient $\left(\frac{Q_{CO_2}}{Q_{O_2}}\right)$ will generally be negative. To think of respiration as a *negative* process is certainly not helpful, and the author prefers to discard the use of algebraic signs in this discussion. This should introduce no misunderstanding so long as the essential nature of the processes is understood.

One other factor needs to be considered in dealing with plant materials, which does not enter into the picture at all in studying animal tissues. Green plant tissues, in the presence of light, carry on the process of photosynthesis, which is the exact reverse of catabolic respiration,

$$6CO_2 + 6H_2O + 674 \text{ Cal.} \underset{\text{Respiration}}{\overset{\text{Photosynthesis}}{\rightleftharpoons}} C_6H_{12}O_6 + 6O_2.$$

This requires very little light, but does require the presence of chlorophyll. In the closed Warburg system this process results in no change in volume, but does alter the proportion of gases available for other reactions. Since this process may go on simultaneously with that of respiration, and since its intensity will vary with the intensity of light impinging on the tissue and with the amount of chlorophyll present (high in internodal tissues, inflorescences, and growing points; moderate in primary, secondary, and graft tumors depending on age; and nil in tumor tissue cultures), it is obvious that the interpretation of results obtained in the light might be completely impossible. Light must be entirely excluded from the respiration flasks during all measurements, and when green tissues are used sufficient time should be allowed in the dark to permit completion of all residual photosynthetic processes before readings are begun. Light was excluded by means of a matt-black hood placed over the water bath and over the manometer heads, with baffles so arranged as to permit shaking and reading the manometers without admitting any traces of reflected light to the flasks.

One deviation was made from the standard procedure, in order that R.Q.'s and Q_{O_2}'s might be obtained on each sample. Instead of running parallel tests with and without KOH and in air and N_2 atmospheres on separate samples, which introduces unpredictable errors due to variations in behavior of different samples, each sample was in most experiments followed for from 1 to 3 hours under each condition without removal from the vessel. Thus, in the most complete type of test, the vessel was first shaken for an hour with tissue and nutrient in air, without KOH, giving a measure of the apparent aerobic R.Q. directly. It was then flushed out for 10 minutes with a stream of N_2, the stopcock closed, equilibrated for 10 minutes, and then shaken for a second hour in an N_2 atmosphere, without KOH, giving a measure of the anaerobic respiration. The flask was then removed and charged with KOH, freshly flushed with N_2, and shaken for a third hour, giving a check on the anaerobic respiration and on the functioning of the apparatus, the theoretical reading being zero. The stopcock was then opened, the flask flushed thoroughly with air, the stopcock closed, and the manometer shaken for a fourth hour with KOH, giving a direct measure of oxygen absorption. From these figures $Q_{O_2}^{Air}$, $Q_{CO_2}^{Air}$, $Q_{CO_2}^{N_2}$,[5] $Q_{N_2}^{N_2}$, and $R.Q.^{Air}$ can all be calculated on the same sample of tissue. This procedure assumes, of course, that previous treatment with one atmosphere does not result in a residual change in behavior in another atmosphere. The experiments show, in fact, that an hour's shaking in N_2, either with or without KOH, does not alter the subsequent shape of the Q_{O_2} curve. The approximately 30 minute period of adjustment involved between procedures while fluids were changed, gases flushed out, final readings recorded, manometers equilibrated, etc., does seem to have been sufficient to eliminate any residual effects, at least within the range of precision of the methods used.

This procedure also introduces certain complications into the calculations. The V_F of tests 1 and 2 differs by 0.5 ml. from that of tests 3 and 4. The solubility correction for tests 1 and 2 involves the summation of $\alpha O_2 + \alpha CO_2$ while that for tests 3 and 4 is dependent on αO_2 only. The appropriate constants calculated to correct for these differences were introduced into the records for each procedure.

Animal respiration studies, especially those carried out in bicarbonate, encounter one problem, the presence of "bound" (dissolved, occluded, or adsorbed) CO_2 in or on tissues or nutrients, which has to be corrected

[5] Anaerobic respiration was measured manometrically only, and was not checked by chemical determination of the metabolic product. Lactic acid determination, the usual criterion in studies of animal tumors, would obviously be meaningless in studies of plant tumors. Determinations of alcohol might or might not prove significant.

for by measurement after freeing with HCl. Experiments with plant tissues, in which bound CO_2 was determined by adding HCl to the tissue-nutrient charge of aliquot vessels at the beginning and at the end of a group of tests, showed that under the experimental conditions used (unbuffered solution containing no carbonate or bicarbonate and very little phosphate) neither nutrient nor tissue contained significant quantities of CO_2. This factor is so small as to be within the experimental error of the method; it can therefore be ignored in all calculations.

Tissue cultures of *Helianthus* tumor tissue, and secondary tumor tissues, were sliced on a microtome to 0.1 mm. thickness. Their respiration was then compared with that of similar cultures torn to a thickness of 1 to 2 mm. The results showed a consistently wider variation in Q_{O_2} values and a lower absolute level of oxygen consumption in sliced than in unsliced samples. The result was interpreted as indicating that tissues containing as great a proportion of large cells as do these, are significantly injured by slicing, reducing their oxygen intake, while oxygen

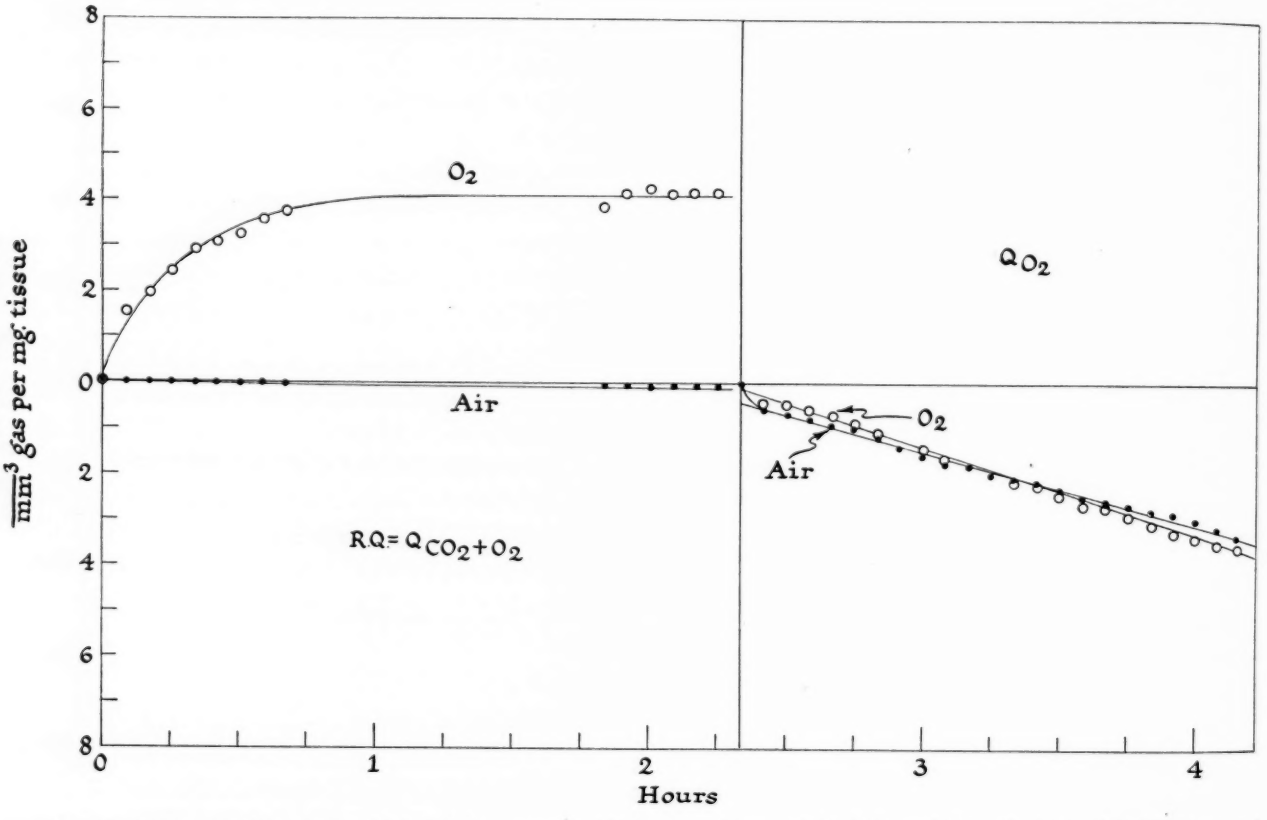

FIG. 1.—Effect of high oxygen (O_2) and low oxygen (Air) atmospheres on the R.Q. and Q_{O_2} of pieces of living tissue cultures of *Helianthus*. The equilibrium slopes for both values are essentially identical in the 2 atmospheres, indicating that maximum *rates* are reached without resorting to increased oxygen levels. This is in distinct contrast to the behavior of most animal tissues.

Respiration studies of animal tissues are usually made on slices of 0.1 mm. or less in thickness and in high-oxygen atmospheres, so as to obtain a maximum oxygen gradient and assure an adequate supply of oxygen to all parts of the tissue. The first procedure involves considerable trauma, which would be even more pronounced with plant tissues (10, pp. 9-11). The second introduces an environment obviously differing radically from that in which metabolism goes on *in vivo*. Both are hence theoretically objectionable, and are used only because of the necessities imposed by the slow diffusion rate of O_2 in animal tissues. Preliminary tests were run to determine if these procedures are likewise necessary for plant tumor materials. diffusion is adequate for the desired interchange without dividing beyond the 1 to 2 mm. limits set by the size of the vessel openings. Tissues were therefore not sliced in the definitive experiments.

This same conclusion was borne out by a second type of test. Tank oxygen was flowed through the vessels containing tissue culture material for 10 minutes so as to introduce a high-oxygen atmosphere. Readings on such vessels were then compared with those on similar vessels containing air (Fig. 1). Cultures in a high-oxygen atmosphere showed a considerable initial increase in total gas volume, resulting in an *apparent* anaerobiosis. The rate of increase was, however, not constant, decreasing rapidly and approaching a zero

value. When a steady rate was reached (after about 2 hours) this rate did not differ significantly from that obtained in air. The rapid initial increase in volume of gas would seem, therefore, to have been referable not to increased CO_2 output, but to release of excess O_2 adsorbed on the tissue or dissolved in the nutrient. There is no indication that increase in oxygen tension actually increases the *rate* of respiration. This result was again interpreted to indicate that oxygen diffusion through unsliced tissues is adequate to support the maximum respiration rate characteristic of these tissues. In definitive experiments cultures were therefore neither sliced nor supplied with a high-oxygen atmosphere.

One feature of these tissues did become evident during the experimentation, which requires attention since it introduces a possible error in the calculation on the basis of the classic Barcroft-Warburg formulae. These formulae are all based on the assumption that the nitrogen of the air, or the pure nitrogen used in measurement of anaerobic respiration, is completely inert and can be ignored. In running tests to check the reliability of the apparatus it became evident that this assumption is not always a valid one. When certain tissues, especially young sunflower inflorescences, are shaken in an atmosphere of pure nitrogen there is a rapid decrease in pressure within the closed system, indicating that nitrogen is absorbed by these tissues. This is not because of the use of impure gas containing traces of oxygen, for the rate of decrease was not reduced when the nitrogen used was first bubbled through concentrated alkaline pyrogallol solution until it no longer darkened pyrogallol in a trap. Approximately the same rate of absorption was also maintained when nitrogen was replaced by helium. This phenomenon thus cannot involve any significant *chemical* reaction. It seems to be purely mechanical. It is possible that nitrogen (or helium) dissolves in the fatty materials in which young inflorescences abound. The rate of absorption at first appears linear, but after 2 to 3 hours' continuous shaking it begins to fall off rapidly, and usually ceases to be significant by the end of 4 or 5 hours. One cannot, however, run each test in a closed atmosphere for 4 or 5 hours *before* beginning to take readings, especially in the presence of a nutrient capable of rapid fermentation if contaminated. We have no data on the exact solubilities of N_2 or He in this nutrient and in the various tissues under investigation. The best that can be done is to run at least a brief empirical test to determine the $Q_{N_2}^{N_2}$ for each sample and then correct the readings of $Q_{CO_2}^{N_2}$ [6] by this amount. Fortunately this absorption is not important with most tissues and is negligible with the tissue cultures, which are perhaps the most important materials under consideration.

[6] We are also faced with the question as to how much absorption goes on in air. If we assume that the nutrient and tissues are saturated with nitrogen at the partial pressure of atmospheric air, a reasonable assumption, then we need make no correction on the $Q_{O_2}^{Air}$, $Q_{CO_2}^{Air}$, and R.Q.^{Air}, but only on the $Q_{CO_2}^{N_2}$. If, on the other hand, we assume that the cuticular layers form a barrier to atmospheric nitrogen so that the internal tissues are at an unsaturated level with respect to atmospheric nitrogen, then a correction would have to be introduced. This last possibility seems extremely unlikely and has been ignored in the following treatment.

EXPERIMENTAL RESULTS

Healthy tissues.—Bacterial tumors, secondary tumors (bacteria-free), and graft (tertiary, metastatic) tumors (also bacteria-free) all are located on the older parts of the sunflower stem, adjacent to healthy internodal tissues. Tissues of the internode including epidermis, cortex, vascular material, and a large proportion of pith are thus *geographically* nearer to the tumor tissues than are any of the more rapidly growing regions such as stem tips. This does not, of course, imply any physiological or anatomical resemblance. For comparative tests segments of internodes from 2 month old sunflower plants were excised, usually about 30 to 40 cm. from the growing point, and split lengthwise into 6 approximately equal sectors. These were cut to such a length as to give wet weights of about 300 mgm. and introduced into the respiration vessels, where possible without further cutting. Four runs, totaling 19 samples, were made. Tests on vegetative stem growing points and on young inflorescences have also been included, since they represent rapidly growing tissues, which may be presumed to bear a closer *physiological* resemblance to tumor tissues than do the slow-growing internodal tissues. The results are illustrated in Fig. 2, top row, and Table I.

The respiratory levels, with the exception of the 2 winter runs (Dec. 11, 1942 and Feb. 5, 1943), were sufficiently high so that the results should possess a fair degree of precision ($Q_{O_2}=3.2$ to 5.5).

The R.Q.'s for internodal tissue (1.1) and young inflorescences (1.1) were not in any way unusual. For stem growing points they were low (0.9), suggesting an anaerobic (incomplete) type of respiration, but the deficiency was not pronounced. The level of anaerobic respiration was low in internodal tissue ($Q_{CO_2}^{N_2}=1.3$) but more than twice as high in meristematic tissue (3.2 for stem growing points and 3.7 for young inflorescences). Evidently healthy meristematic tissues are *capable* of a much higher anaerobic respiration than are internodal tissues, although their aerobic respiration levels were about the same.

Diseased tissues; bacterial galls.—Although the tis-

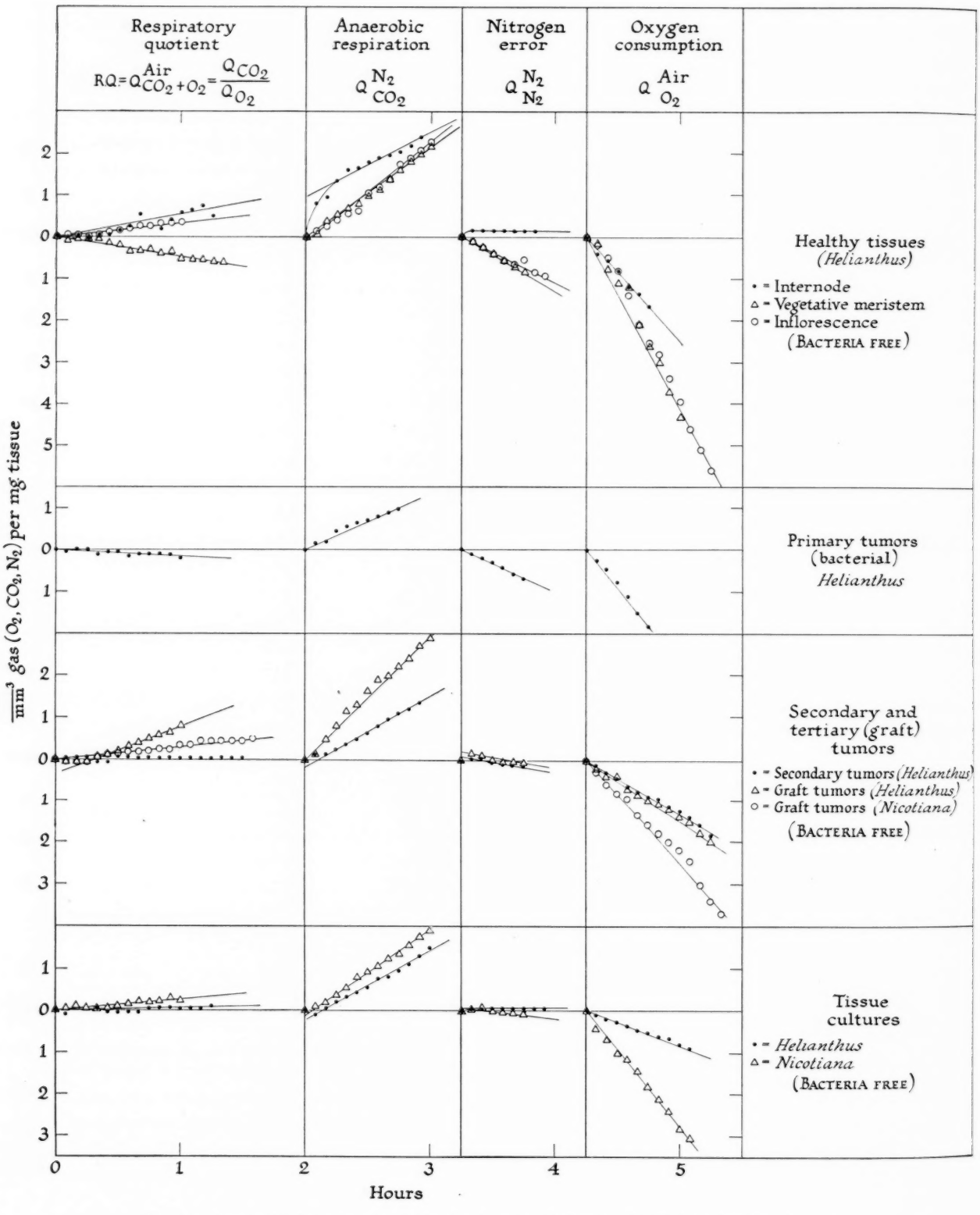

FIG. 2.—Summarized respiratory results for 9 types of tissues. Detailed explanation in text.

sues in which we are primarily interested, those of secondary and tertiary (metastatic) galls and tissue cultures derived therefrom, are all bacteria-free, the existence of such tumor tissues is referable in some as yet obscure fashion to the interaction of healthy host tissues with actively metabolizing colonies of *Phytomonas tumefaciens*. The behavior of these bacteria-free tumor tissues is therefore to be compared not only with healthy host tissues but also with the bacteria-infested tissues of primary crown-galls. A single series of tests was made of the respiratory processes in tissues of rapidly growing galls arising on young sunflower plants at and adjacent to the loci of inoculation with a virulent culture of *Phytomonas tumefaciens*. The results are summarized in Fig. 2, second row, and Table II.

Here the respiratory level was surprisingly low, the $Q_{O_2}^{Air}$ being only 3.7. This is the same as that for slowly growing healthy internodal tissue (3.7), and considerably below that for healthy meristems (4.5 and 5.5). The aerobic and anaerobic respiration was about the same as that for healthy meristems. The R.Q. was somewhat lower than for most healthy tissues (0.9), but probably not significantly so. The presence of active bacterial colonies does not appear to alter the respiratory picture qualitatively to any significant degree, so far as can be shown by the methods employed here. The only change is a quantitative one, a reduced respiratory level.

Bacteria-free tumor tissues.—Three types of bacteria-free tumor tissues of sunflower were studied: (a) secondary galls, (b) tissue cultures, and (c) graft tumors (tertiary, metastatic). Results with these and with comparable tissues from hereditary tumors of *Nicotiana* are presented in Fig. 2, third and fourth rows, and Table III.

Here the respiratory level was again in all cases low, the value of 3.6 for *Nicotiana* graft tumors being the highest. The respiratory quotients were normal, with the exception of that for graft tumors of sunflower (1.3), which may be due to an excess of necrotic tissue. Anaerobic respiration was fairly low except in the March 5 and 11 runs on sunflower graft tumors, and even here it did not equal the values for healthy growing tissues of sunflower. Nowhere does the picture appear to be clearly out of the ordinary except, again, as regards *level* of activity.

TABLE I: RESPIRATION OF HEALTHY SUNFLOWER TISSUES

Date	Tissue	Percentage dry wt.	Number tested	$Q_{O_2}^{Air}$ * Aerobic oxygenation			$Q_{CO_2}^{Air}$ Aerobic respiration			$Q_{CO_2}^{N_2}$ Anaerobic respiration				$Q_{N_2}^{N_2}$ Nitrogen correction			R.Q. Respiratory quotient		
				Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	Corr. Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dec. 11, 1942	Internodes	8.1 (3.6-12.9)	6	1.8	0.4	1.0	2.0	0.5	1.1								1.3	1.1	1.2
Feb. 5, 1943			5	1.3	0.9	1.1	1.2	0.7	1.0								1.1	0.8	0.9
Sept. 30, 1943			4	5.6	2.8	4.2	5.3	3.2	4.1	1.6	0.4	0.8	0.8	0.0	0.0	0.0	1.1	0.9	1.0
Oct. 6, 1943			4	4.1	2.5	3.2	4.9	3.0	3.8	2.9	0.0	1.8	1.8	0.1	0.0	0.0	1.3	1.1	1.2
Averages			19	3.2	1.4	2.4	3.6	1.9	2.5	2.2	0.2	1.3	1.3	0.0	0.0	0.0	1.2	1.0	1.1
Mar. 19, 1943	Stem tips	10.5 (8.0-15.0)	6	4.9	3.1	3.8	4.7	2.2	3.2	3.1	1.8	2.2	2.6	0.7	0.0	0.4	0.9	0.7	0.8
Nov. 18, 1943			6	6.3	3.9	5.2	5.9	3.5	4.6	2.8	1.7	2.2	3.8	2.3	1.0	1.6	0.9	0.8	0.9
Averages			12	5.6	3.5	4.5	5.3	2.8	3.8	2.9	1.8	2.2	3.2	1.5	0.5	1.0	0.9	0.8	0.9
Feb. 24, 1943	Young inflorescences	11.6 (9.7-19.0)	6	6.6	4.2	5.5	6.8	4.5	5.3	4.3	1.3	2.4	3.7	1.8	1.2	1.4	1.2	1.0	1.1

* See Footnote 2.

TABLE II: RESPIRATION OF PRIMARY CROWN-GALL TISSUES OF SUNFLOWER CONTAINING ACTIVE COLONIES OF *Phytomonas tumefaciens*

Date	Tissue	Percentage dry wt.	Number tested	$Q_{O_2}^{Air}$			$Q_{CO_2}^{Air}$			$Q_{CO_2}^{N_2}$				$Q_{N_2}^{N_2}$			R.Q.		
				Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	Corr. Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dec. 9, 1943	Primary tumor	11.6 (8.8-15.0)	6	4.6	3.2	3.7	4.1	3.0	3.4	1.6	1.1	1.4	2.8	1.6	1.2	1.4	1.0	0.9	0.9

DISCUSSION

The results of all these tests are summarized in Table IV. The respiratory level was high in healthy meristems (growing regions); intermediate in tissues containing bacteria and in the hereditary tumors of *Nicotiana,* both *in vitro* and *in vivo;* and low in bacteria-free crown-gall tissues in whatever form. If a result, however, of the excess of woody and necrotic tissues in these tumors. Anaerobic respiration was high in healthy meristems, but not decidedly so in any type of diseased tissue. This characteristic appears to be bound up not with the tumorous state, but with actively growing meristem. Absorption of nitrogen is an important factor requiring correction in healthy

TABLE III: RESPIRATION OF BACTERIA-FREE TUMOR TISSUES

Date	Tissue	Percentage dry wt.	Number tested	$Q^{Air}_{O_2}$			$Q^{Air}_{CO_2}$			$Q^{N_2}_{CO_2}$				$Q^{N_2}_{N_2}$			R.Q.		
				Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	Corr. Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dec. 2, 1943	Secondary tumors		5	2.6	1.1	1.8	2.8	1.1	1.9	1.9	0.9	1.4	1.7	0.6	0.1	0.4	1.1	1.0	1.0
Oct. 21, 1943	Tissue cultures, sunflower	7.3 (3.3-12.9)	5	4.0	1.5	2.4	4.4	1.3	2.4	2.4	1.7	2.1	2.3	0.8	0.0	0.2	1.3	0.8	1.0
Oct. 28, 1943			6	1.2	0.8	1.0	1.2	0.9	1.1	1.8	1.2	1.5	1.5	0.1	0.1	0.0	1.3	1.0	1.1
Nov. 4, 1943			5	1.8	0.9	1.2	2.1	1.0	1.6	2.3	1.3	2.0	2.0	0.1	0.0	0.0	1.8	1.0	1.2
Averages			16	2.3	1.1	1.5	2.6	1.0	1.7	2.2	1.4	1.8	1.9	0.2	0.0	0.0	1.4	0.9	1.1
Nov. 11, 1943	Tissue cultures, *Nicotiana*	8.7 (5.4-15.8)	6	5.0	1.9	3.7	5.3	1.9	3.8	2.8	1.1	1.9	2.1	0.7	0.0	0.2	1.1	1.0	1.0
Jan. 20, 1944			6	2.9	1.8	2.3	3.0	1.9	2.5	1.3	0.8	1.1	1.7	0.9	0.4	0.6	1.1	1.0	1.1
Averages			12	4.0	1.9	3.0	4.2	1.9	3.2	2.0	1.0	1.5	1.9	0.8	0.2	0.4	1.1	1.0	1.1
Jan. 13, 1943	Graft tumors, sunflower	11.8 (8.4-17.1)	6	1.5	0.8	1.3	1.4	0.7	1.1	0.7	0.4	0.5	0.8	0.4	0.2	0.3	1.1	0.5	0.9
Mar. 5, 1943			5	2.5	1.0	1.6	3.8	2.1	2.6	4.5	3.2	3.8	4.1	1.5	0.2	0.3	2.1	1.4	1.7
Mar. 11, 1943			5	2.7	1.4	2.1	3.6	2.6	3.1	3.8	2.4	3.2	3.4	0.3	0.1	0.2	1.8	1.1	1.5
Dec. 2, 1943			5	2.6	1.1	1.8	2.8	1.1	1.9	1.9	0.9	1.4	1.7	0.6	0.1	0.4	1.1	1.0	1.0
Averages			21	2.3	1.1	1.7	2.9	1.6	2.1	2.7	1.7	2.2	2.5	0.7	0.1	0.3	1.5	1.0	1.3
July 14, 1943	Graft tumors, *Nicotiana*		6	5.0	2.2	3.6	5.0	2.7	3.9								1.2	1.0	1.1

TABLE IV: SUMMARY OF RESPIRATORY BEHAVIOR OF TISSUES OF TUMOR-BEARING PLANTS OF *Helianthus* AND *Nicotiana*

Tissue	$Q^{Air}_{O_2}$	$Q^{Air}_{CO_2}$	$Q^{N_2}_{CO_2}$	$Q^{N_2}_{N_2}$	R.Q.
Healthy internodal tissue	3.7	3.9	1.3	0.0	1.1
" stem-tips	4.5	3.8	3.2	1.0	0.9
" inflorescence tissue	5.5	5.3	3.7	1.4	1.1
Primary (bacterial) gall	3.7	3.4	2.8	1.4	0.9
Secondary (bacteria-free) gall	1.8	1.9	1.7	0.4	1.0
Tertiary (graft, bacteria-free) gall, sunflower	1.7	2.1	2.5	0.3	1.3
" " " " " *Nicotiana*	3.6	3.9	*	*	1.1
Tissue culture (bacteria-free), sunflower	1.5	1.7	1.9	0.0	1.1
" " " " *Nicotiana*	3.0	3.2	1.9	0.4	1.1

* Not determined.

this low respiratory level is real and not referable to the amount of woody or necrotic (non-respiring) material present in the samples—and tests based on protein nitrogen instead of dry weight determinations should answer this question, then it is important, and agrees with the Burk-Warburg idea that respiratory derangement or deficiency is characteristic of tumors. The respiratory quotients were normal (0.9 to 1.1) in all tissues with the exception of the graft tumors of sunflower, where the R.Q. was 1.3. This also may be inflorescence tissue and in primary crown-galls (both rapidly growing), but not in other tissues. Although such qualitative deviations from normal as do occur *may* be indicative of real and significant abnormalities in respiratory behavior, it seems more probable that they are to be attributed to differences in denseness of tissue, rate of gas diffusion, and similar factors, and to the inaccuracies inherent in the experimental method when applied to materials having a low respiratory level, rather than to any qualities of tumor

tissues as opposed to healthy tissues. The tumefacient change that these tissues have undergone does *not* express itself in any qualitative change in the respiratory picture that is clearly distinguishable by this method of study. Other methods may of course reveal such changes but, so far as one can distinguish by this particular use of the Barcroft-Warburg method, the respiratory process has not been altered in kind. This conclusion agrees with that arrived at by Burk and his associates (3) in their studies of chicken tumors.

The work presented here is admittedly incomplete, since the writer has had no previous experience or training in the investigation of problems of this nature. Nevertheless, it is hoped that it may serve to stimulate others of greater competence to see what more can be extracted from the study of respiratory processes in plant tumors.

SUMMARY

Studies by the Barcroft-Warburg method on the respiration of healthy vegetative growing points, inflorescences, and internodes of *Helianthus annuus;* of pathological tissues of the same plant represented by crown-gall tumors containing active colonies of *Phytomonas tumefaciens*, secondary tumors free of bacteria, tertiary (metastatic, graft) tumors arising as a result of implantation of bacteria-free tumor tissue cultures under the bark of healthy plants, and tissue cultures derived from bacteria-free secondary tumors; of genetically tumefacient tissues of the hybrid, *Nicotiana langsdorffii* × *N. glauca,* tissue cultures derived from the meristem of this hybrid, and tumors arising as a result of implantation of such tissue cultures under the bark of *Nicotiana glauca* have led to the conclusion that these various pathological states do not result in any apparent significant *qualitative* change in the respiratory picture, but do result in a definite lowering of the respiratory *level.* If this lowering is real and not merely an artefact due to the greater amount of nonliving tissue present in pathological growths, it may be considered similar in kind to long recognized characteristics of animal neoplasia.

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Abstracts

Reports of Research

Influence of Age on Epidermal Carcinogenesis Induced by Methylcholanthrene in Mice. COWDRY, E. V., and SUNTZEFF, V. [Barnard Free Skin and Cancer Hosp., and Washington Univ., St. Louis, Mo.] *Yale J. Biol. & Med.*, **17**:47-58. 1944.

Under the experimental conditions specified, tumors induced by methylcholanthrene appeared more quickly and in a higher percentage of individuals in young than in old New Buffalo mice—a difference not observed between young and old CBA mice. Young New Buffalo mice developed tumors more promptly and in a higher percentage of individuals than young CBA mice, but this difference between strains was not observed in the older animals. The initial strain difference in susceptibility disappeared with advancing age.—J. L. M.

The Carcinogenicity of Certain Azo Dyes Related to *p*-Dimethylaminoazobenzene. MILLER, J. A., and BAUMANN, C. A. [Univ. of Wisconsin Coll. of Agric., Madison, Wis.] *Cancer Research*, **5**:227-234. 1945.

Eleven compounds closely related to *p*-dimethylaminoazobenzene were fed to rats at levels equivalent to 0.06% of this dye in an adequate semisynthetic diet for periods up to 240 days, and their potencies as liver carcinogens noted up to 300 days. *m'*-Methyl-*p*-dimethylaminoazobenzene proved to be more active than *p*-dimethylaminoazobenzene. *p*-Monomethylaminoazobenzene was practically as active as the parent compound. *o'*-Methyl-*p*-dimethylaminoazobenzene and *p'*-methyl-*p*-dimethylaminoazobenzene in that order were less active than *p*-dimethylaminoazobenzene. The remaining 7 compounds, *p*-aminoazobenzene, *o*-methyl-*p*-dimethylaminoazobenzene, *p*-dimethylamino-phenylazo-1-naphthalene, N,N-dimethyl-N'-benzal-*p*-phenylenediamine, *p*-dimethylaminobenzal-aniline, N,N,-dimethyl-*p*-phenylenediamine, and aniline, were inactive under the conditions employed.

Aqueous solutions of the hydrochlorides of *p*-phenylenediamine and its N-monomethyl and N,N-dimethyl derivatives were found to give characteristic stains on pine chips. By this test rats fed the split product of *p*-dimethylaminoazobenzene, N,N-dimethyl-*p*-phenylenediamine, were found to excrete some of this diamine as such in the urine. No similar excretion of chromogen was noted in rats fed the azo dye. Each of the *p*-dimethylaminoazo dyes tested underwent a stepwise demethylation *in vivo* prior to cleavage at the azo linkage.

The new data fail to support the theory of Kensler, Dexter, and Rhoads on the mechanism by which the azo dyes produce liver tumors.—Authors' abstract.

Observations on Rats Fed with Compounds Related to Dimethylaminoazobenzene. SUGIURA, K., HALTER, C. R., KENSLER, C. J., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] *Cancer Research*, **5**:235-238. 1945.

The carcinogenic action of several new compounds of the series of dimethylaminoazobenzene was tested in the rat by oral administration. All rats fed 4.8 mgm. of N,N-dimethyl-*p*-aminoazobenzene daily for a period of 150 days developed liver cancer. Daily ingestion of 4.8 mgm. of N,N-diethyl-*p*-aminoazobenzene, N,N-dipropyl-*p*-aminoazobenzene, N,N-dibutyl-*p*-aminoazobenzene, or N,N-diamyl-*p*-aminoazobenzene failed to produce liver cancer, thus indicating the importance for carcinogenesis of the methyl radical in the chemical structure. Although N-methyl-*p*-aminoazobenzene is highly carcinogenic, its isomer 4'-methyl-4-aminoazobenzene is only feebly so. Daily ingestion of 2',3',4',5',6'-pentamethyl-4-aminoazobenzene, *p*-sulfanilylamidodimethylaniline, N,N-dibutyl-*p*-phenylenediamine, diacetyl-*p*-phenylenediamine (a metabolite of N,N-dimethyl-*p*-aminoazobenzene), and combined feeding of N,N-dimethyl-*p*-phenylenediamine and aniline (split products of N,N-dimethyl-*p*-aminoazobenzene), failed to produce liver cirrhosis or tumors in rats.—Authors' abstract.

The Effects of Plant Growth Substances Upon the Metabolism of Yeast. BAIN, J. A., and RUSCH, H. P. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Arch. Biochem.*, **5**:317-328. 1944.

The effects of plant growth substances; of *p*-phenylenediamine and dimethyl-*p*-phenylenediamine, which are split products of the carcinogenic dye, *p*-dimethylaminoazobenzene; and of naphthoquinone and quinone upon the metabolism of yeast were investigated. The systems that oxidize pyruvate were found to be most sensitive. Next in order of sensitivity were those enzymes concerned in the oxidation of glucose. Least sensitive were the systems involved in the anaerobic utilization of glucose. These results are interpreted as indicating that a differential inhibition of aerobic systems of metabolism could take place under the influence of these inhibitors, and such a possibility is discussed with reference to the problem of cellular proliferation.—Authors' abstract.

The Nucleic Acid and Nucleotide Content of Tumours. DAVIDSON, J. N., and WAYMOUTH, C. [Univ. of Aberdeen, Aberdeen, Scotland] *Brit. J. Exper. Path.*, **25**:164-173. 1944.

The tumors employed for this investigation were the Rous sarcoma and a chemically induced fowl sarcoma (Peacock's GRCH 15), also a variety of human tumors (both sarcomas and carcinomas). All the human material was freshly obtained at biopsy in order to eliminate the possibility of postmortem conversion of nucleotides to

nucleosides. In confirmation of previous work the water content of all tumors was found to be high. With the more cellular tumor tissue the total nucleic acid content (desoxyribonucleic and ribonucleic acids) was high. In this respect and in the low concentrations of acid-soluble nucleotides, tumor tissue resembled the highly cellular tissues of the rapidly growing embryo. The blood of fowls with the Rous sarcoma showed an increase of polypeptide nitrogen and nonprotein nitrogen above the normal. The levels of both were raised above the normal mammalian blood values.—R. J. L.

Untersuchungen über den Einfluss von *d*- und *d,l*-Di- und Polypeptiden auf die Entwicklung von Impftumoren. [Investigations on the Influence of *d*- and *d,l*-Di- and Polypeptides on the Development of Transplanted Tumors.] ABDERHALDEN, R. [Physiol. Inst., Martin Luther Univ., Halle, Germany] *Ztschr. f. physiol. Chem.*, **270**:9-13. 1941.

Waldschmidt-Leitz and his associates (*ibid.* **263**:1. 1940; **267**:79. 1941) state that they have inhibited notably the development of benzpyrene carcinoma in mice by intravenous injections of a racemic dipeptide (*d,l*-glutaminylglycine). This treatment was based on Kögl's theory of tumor genesis, involving "unnatural" amino acid isomers of the *d*-series, and on preceding reports by Waldschmidt-Leitz and his group, according to which the administration of racemic di- and polypeptides produces the formation of *d*-peptidases. The latter would then be expected to act like "abwehrfermente," which would prevent the synthesis of abnormal proteins containing *d*-amino acids. The present author reinvestigated these claims by treating rats with *d*-alanylglycylglycine, *d,l*-leucylglycylglycine, and *d,l*-leucylglycine subcutaneously before and after inoculation with Walker and Flexner carcinomas. No difference was noted in the growth of these transplanted tumors in the treated animals and the untreated controls. In order to facilitate the entry of the active substances into the tumor tissue, another series of experiments was conducted on mice inoculated intraperitoneally with Ehrlich carcinoma, which under these conditions produces the Ehrlich "ascites tumor" in which the tumor cells grow suspended in a transudate into the peritoneum. The tumor growth in this case can be followed by periodic weighing of the animals; the growth rate of the tumor is a function of the number of inoculated tumor cells. Again no difference was seen between the controls and the animals treated with peptides for several weeks in advance of the inoculation of tumor cells.—K. G. S.

Degradation of Cystine Peptides by Tissues. I. Exocystine Desulfurase and Dehydropeptidase in Rat-Liver Extracts. GREENSTEIN, J. P., and LEUTHARDT, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:209-221. 1944.

The enzyme "desulfurase," which degrades cystine as well as certain cystine-containing peptides with the formation of hydrogen sulfide, pyruvic acid, and ammonia, has been found to occur in certain normal tissues (liver, kidney, pancreas), while it is completely absent from the tumor tissues examined for it. As the source of enzyme in the present investigation crude, normal rat liver extracts were employed. The same extracts contain a dehydropeptidase. The authors advance the theory that the cystine peptides are degraded by these extracts in a sequence of reactions consisting of: (1) an enzymatic desulfuration by "exocystine desulfurase," yielding hydrogen sulfide (and sulfur) and the corresponding unsaturated dehydropeptide, (2) the enzymatic hydrolysis of the latter by the dehydropeptidase to aminoacrylic acid, and (3) the spontaneous hydrolysis of the labile aminoacrylic acid to form pyruvic acid and ammonia.—K. G. S.

Degradation of Cystine Peptides by Tissues. II. Distribution of Exocystine Desulfurase and Dehydropeptidase in Tissue Extracts of Various Species. GREENSTEIN, J. P., and LEUTHARDT, F. M. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:223-225. 1944.

According to the scheme indicated in the preceding paper (see preceding abstract), 2 enzymes, namely, exocystine desulfurase and dehydropeptidase, cooperate in the breakdown of cystine peptides to hydrogen sulfide, pyruvic acid, and ammonia. In order to ascertain whether the activity of these 2 enzymes runs parallel in different sources, the ability of crude extracts of liver, kidney, pancreas, spleen, brain, and muscle tissue, from rats, mice, rabbits, and guinea pigs, to liberate ammonia from *l*-cystine, dichloracetyl-*l*-cystine, and chloracetyldehydroalanine was examined. Liver, kidney, and pancreas tissue, in contrast to the other tissues tested, were found to possess such enzymatic activity. It is concluded that the 2 enzymes in question either occur together or (in other tissues) do not occur at all.—K. G. S.

The Degradation of Cystine Peptides by Tissues. LEUTHARDT, F. M., and GREENSTEIN, J. P. [Nat. Cancer Inst., Bethesda, Md.] *Science*, **101**:19-21. 1945.

Previous work by Bergmann showed that amino acids might be enzymatically dehydrogenated while still in peptide combination, and that the resulting dehydropeptides could be hydrolyzed by a dehydropeptidase present in tissue extracts. The present authors develop this system further by suggesting that peptides of cystine (or cysteine) are the natural precursors of the dehydropeptides (see 2 preceding abstracts).—R. B.

Occurrence of Follicular Cysts in Thyroidectomized Rats Treated with Diethylstilbestrol. JANES, R. G. [Wayne Univ. Coll. of Med., Detroit, Mich.] *Anat. Rec.*, **90**:93-99. 1944.

Removal of the thyroid from Long-Evans rats resulted, after 8 months, in the development of cystic ovaries in 1 of 8 animals. If, in addition, the rats were injected at the end of this period with daily doses of 100 μgm. of diethylstilbestrol for 20 days, the number of animals with cystic ovaries increased to 7 out of 10. On the other hand, continuous administration of diethylstilbestrol for 8 months, by means of implanted pellets, usually resulted in atrophy of the ovaries in both normal and thyroidectomized rats.—R. B.

On the Behavior of Fibromatogenic Quantities of Alpha-Estradiol and its Esters Absorbed from Intrasplenic Tablets. LIPSCHÜTZ, A., and ACUNA, L. [Nat. Health Service, Santiago, Chile] *Rev. canad. de biol.*, **3**:96-107. 1944.

Intrasplenically implanted tablets of α-estradiol and of

dipropionic and 17-caprylic esters of estradiol did not induce the formation of abdominal fibroids in 30 of 32 female guinea pigs observed over a period of 61 to 70 days. The quantities of α-estradiol and its esters absorbed daily from these intrasplenic tablets were comparable to the fibromatogenic quantities absorbed from subcutaneously implanted tablets. In 2 animals, receiving the dipropionate, uterine and extragenital fibroids appeared in the course of 2 months. This is explained by the fact that in each case the tablet came to the surface of the spleen and hence into immediate contact with the abdominal cavity. Not all the free or esterified hormone absorbed from the spleen was completely inactivated in many of the animals in which fibroids failed to appear. The condition of the vaginal mucosa and of the uterine wall indicated that estrogenic and hysterotrophic quantities of the hormone escaped inactivation until the end of the experiments. This was probably because of the passage of a certain amount of hormone into the general circulation through splenic adhesions.—C. A.

Hepatic Autodefense against the Tumorigenic Action of Estrogens. Lipschütz, A., and Carrasco, R. [Nat. Health Service, Santiago, Chile] *Rev. canad. de biol.*, **3**:108-125. 1944.

α-Estradiol, benzoic and 17-caprylic esters of estradiol, and estrone tablets were implanted intrahepatically in 83 guinea pigs and subcutaneously in 47. Castrated female guinea pigs of 340 to 680 gm. were used. The quantities of hormone absorbed daily in each group were the same.

Abdominal and uterine fibroids were induced in each group, but the tumor incidence was 70% less in animals with intrahepatic tablets than in those with subcutaneous tablets. In 30% of the animals with intrahepatic tablets (generally those with the larger tablets containing estradiol esters) perihepatic adhesions were found. Only in rare exceptions, however, did these animals present a fibrous reaction equal to that seen with subcutaneously implanted tablets. These results are fundamentally different from those with intrasplenic tablets, after which fibroids never occurred. Uterine epitheliotoxic reactions such as cystic glandular hyperplasia were present in all animals examined, in contrast to the results with intrasplenic tablets.

It is assumed that estrogen absorbed in the spleen and carried by the portal vein is put in contact with the entire liver at a low concentration, whereas estrogen absorbed in the liver establishes contact with a limited part of the organ and in higher concentrations. This would indicate that the inactivating antiestrogenic faculty or the autodefensive activity of the liver is quantitatively limited.—C. A.

The Induction of Mammary Carcinoma in the Rat. Nelson, W. O. [State Univ. of Iowa, Iowa City, Iowa] *Yale J. Biol. & Med.*, **17**:217-228. 1944.

The administration of estrogenic substances by injection or by implantation of pellets in rats induced the appearance of mammary carcinoma in 68 of 103 animals that survived treatment long enough to permit the development of cancer (at least 300 days). It seems probable that some animals are resistant to the final stages of the neoplastic process which leads to the development of mammary carcinoma.

The various cases were distributed among several types of carcinoma. Duct carcinoma, 42 rats; adenocarcinoma, 8 rats; mixed duct and adenocarcinoma, 13 rats; carcinoma simplex, 5 rats. Metastases to lymph nodes or to lymph nodes and lungs were observed in 33 animals.—Author's summary. (J. L. M.)

The Leukemogenic Action of Estrogens in Hybrid Mice. Gardner, W. U., and Dougherty, T. F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Yale J. Biol. & Med.*, **17**: 75-90. 1944.

Of 1,403 estrogen-treated mice 165, or approximately 12%, died with lymphoid tumors, and 34, or 4.5%, of 739 control mice died with tumors of the same type. Hybrid mice born of parents of 2 strains susceptible to estrogen-induced lymphomas had a high incidence of such tumors, 40 and 45%, while no tumors occurred in the untreated controls. Hybrid mice born of parents 1 of which was susceptible to estrogen-induced leukemia and 1 resistant showed incidences intermediate between the parental stocks. Hybrid mice born of parents from 2 strains resistant to estrogen-induced leukemias had in 1 instance higher and in 1 instance lower incidences of lymphomas than the parental strains. The incidence of spontaneous lymphomas among the untreated mice was high in 3 groups (11.5 to 20%). Among the mice of these groups the incidence of myeloid metaplasia was also high, and 2 mice showed erythroblastic neoplasia. The lymphoid tumors were of the same types as described as occurring among mice of the parental inbred strains.—Authors' summary. (J. L. M.)

Recent Studies on Experimental Mammalian Leukemia. Kirschbaum, A. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Yale J. Biol. & Med.*, **17**:163-187. 1944.

A review of the literature and of the investigations of the author, with a 4 page bibliography. The following topics are discussed: the nature of mouse leukemia; its induction by carcinogens, estrogens, x-rays, and benzol; genetics of mouse leukemia; nongenetic influences on spontaneous leukemia (maternal influence, diet, endogenous hormones); transplantation studies; metabolism of mouse leukemia; experimental therapy.—J. L. M.

Leukemia in Mice Following Exposure to X-Rays. Henshaw, P. S. [Nat. Cancer Inst., Bethesda, Md.] *Radiology*, **43**:279-285. 1944.

Of 57 black mice (C57) given whole body radiation of 200 r at intervals of 4 weeks for a period of 20 weeks, 30% developed leukemia, as compared to 7% among the nonirradiated controls. The blood leukocyte level dropped during radiation and thereafter slowly rose to normal; leukocytosis invariably accompanied the leukemia.—R. E. S.

The Influence of Irradiation of the Ovaries upon Estrus and Neoplastic Development in Marsh-Buffalo Mice. Bischoff, F., Ullmann, H. J., and Ingraham, L. P. [Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.] *Radiology*, **43**:55-58. 1944.

Carefully controlled groups of 40 mice each were given 200 and 400 r to the ovaries after the establishment of vaginal canalization. The 200 r markedly reduced estrus immediately after irradiation, but in the fourth month

thereafter there was an increase in estrus; 400 r reduced estrus entirely, and it was never re-established as normal during the 13 months of observation. The incidence of adenocarcinoma of the breast was increased by 200 r, not by 400 r, while the incidence of lymphoid tumors was increased by 400 r, not by 200 r.—R. E. S.

Mammary Tumour Inducing Factor and Genetic Constitution. Dmochowski, L. [Lab. of Imp. Cancer Research Fund, Mill Hill, England] *Brit. J. Exper. Path.*, **25**:138-140. 1944.

"S" low cancer strain mice were foster nursed by females of the RIII high cancer strain, and their spleens were grafted into low tumor hybrid S×RIII mice. Two of the mammary tumors that ultimately developed were desiccated *in vacuo,* and after 2 weeks' storage resuspended in distilled water and injected into hybrids from C57 females × Strong A males. A number of these hybrids when approximately 5 weeks old had been marked individually and divided into 3 groups. The females of the first group were injected with dried tumors from the S×RIII mice and were then forcibly bred. Those of the second group received no tumor injections but were forcibly bred, while those of the third group were allowed to breed and bring up their litters in the normal manner. Mammary tumors developed in 7 of 11 mice of the first group (64%), while in the 21 mice in the other 2 groups there was no case of cancer. The mammary tumor inciter is therefore capable of inducing breast cancer in mice of a different genetic constitution from that of the strain from which it originated, even after a preliminary transmission through mice of another genetic constitution.—R. J. L.

Survival of the Mammary Tumor Milk Agent of Mice. Bittner, J. J., Evans, C. A., and Green, R. G. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Science,* **101**: 95-97. 1945.

The mammary tumor milk agent survived in C3H mammary carcinoma during 10 serial passages in mice that did not themselves originally carry the agent. It also survived 12 days in the yolk sac of chick embryos after injection of macerated mammary tumor tissue or of cell-free filtrates of mammary tumors. In both groups of experiments the survival of the agent was demonstrated by injecting the test material into ABC or ZBC hybrid mice. These animals do not carry the agent but are sensitive to it. One-third or more of the injected animals developed mammary cancer, while among the controls the incidence was less than 1%.—R. B.

Genetic Nature of the Constitutional States of Cancer Susceptibility and Resistance in Mice and Men. Strong, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] *Yale J. Biol. & Med.*, **17**:289-299. 1944.

In an attempt to duplicate or imitate in experimental animals the variable genetic background of man, hybridization of mice and selection toward resistance to methylcholanthrene-induced local tumors were effected. Under these controlled experimental conditions a great number of cancers were produced in mice. In fact, the mice gave rise to a very high incidence of multiple malignancies. The data indicate, therefore, that biological variability, as determined by genetic determiners, is responsible for the various types of cancer found in mice and possibly in man also. The production of the selected NHO strain of mice now makes available for further research under controlled conditions in experimental animals a wealth of new neoplastic diseases, one of which, namely cancer of the stomach, will unquestionably be of especial value in view of the prevalence of this type of cancer in man.—Author's summary. (J. L. M.)

Les Facteurs Constitutionnels du Cancer Étudiés sur des Rats par la Méthode de la Parabiose. [A Study of the Constitutional Factors of Cancer by Means of Parabiosis.] Foa, C., and Monteiro, U. [São-Paulo Univ., São-Paulo, Brazil] *Rev. canad. de biol.*, **2**: 259-270. 1943.

A spontaneous angioendothelioma in one of a pair of parabiotic rats is reported. Two female rats, belonging to different families and to mixed strains, were joined together at an early age in a way permitting free communication between their peritoneal cavities. After living 1 year in perfect nutritive equilibrium, they died without having presented any previous symptoms. At autopsy an abdominal angioendothelioma with numerous visceral metastases was found in one rat, while the other was completely devoid of neoplastic growths. The authors conclude that this casual observation supports the idea that the individual organism is a fundamental factor in tumor resistance.—C. A.

Production of 'Endomitosis' in Bean Roots and its Bearing on the Genesis of Tumours. Mottram, J. C. [Mt. Vernon Hosp., Northwood, Middlesex, England] *Nature, London,* **154**:828. 1944.

Endomitosis has been induced in bean roots by (1) exposure to benzpyrene, (2) exposure to hypertonic sugar solution, and (3) partial drying. Since (2) and (3) increase the viscosity of cytoplasm, and since tumor cells are reported to have a high cytoplasmic viscosity, it is suggested that endomitosis in tumors and also the occurrence of polyploidy and polynuclear cells are "secondary to an increased stiffness of the cytoplasm and no more than the signs of such a change."—R. J. L.

The Production of Multipolar Mitoses in Normal Somatic Cells of Embryonic Chick Tissue Grown *in vitro*. Stilwell, E. F. [Woman's Med. Coll. of Pennsylvania, Philadelphia, Pa.] *Anat. Rec.*, **90**:115-131. 1944.

Embryonic chick heart fibroblasts growing in culture exhibited many more multipolar mitoses than are ordinarily found among these cells. These aberrant division figures resembled those found in certain neoplasms. A number of variables in the tissue culture set-up were discussed as possible factors contributing to the production of the abnormal mitoses.—R. B.

A Method of Obtaining Tissue Cultures of Adult Fibroblasts. Peacock, P. R., and Shukoff, R. I. [Glasgow Roy. Cancer Hosp., Glasgow, Scotland] *Nature, London,* **154**: 799. 1944.

In the course of experiments upon the virus of Rous sarcoma, the authors devised a technic for obtaining tissue cultures in the form of a fibrin clot invaded by fibroblasts and macrophages, by introducing glass capillary tubes into the breast muscles of fowls by means of a trocar and cannula.—E. L. K.

Concurrence of Growth-Promoting and Growth-Inhibiting Factors in Extracts of Adult Rat Tissues. WERNER, H. [Hebrew Univ., Jerusalem, Palestine] *Nature, London,* **154**:827. 1944.

Saline extracts of rat heart muscle either have no stimulating action or may inhibit cell growth *in vitro,* but a growth-promoting substance has been prepared as follows: Minced heart muscle was extracted with 4 volumes of normal saline, and this extract was precipitated with 4 volumes of alcohol. The precipitate obtained was then treated in a Soxhlet apparatus with acetone or petroleum ether. The extracted material, after drying, was taken up in Tyrode solution. The growth of chick fibroblasts in Carrel flasks with this solution as supernatant fluid phase was 4½ times as great as that of control cultures in which the fluid phase was Tyrode solution. It is therefore concluded that the inability of rat tissue extracts to stimulate growth of cells *in vitro* is the result of the suppression of the growth-promoting substance of the heart muscle by the predominance of growth-inhibiting factors, and that the latter are probably of a lipoid nature.—R. J. L.

The Effects of Gastric Mucin on the Growth of Transplanted Tumor (Lymphosarcoma) Cells in Mice. WILLIAMS, W. L. [Yale Univ. Sch. of Med., New Haven, Conn., and Louisiana State Univ. Sch. of Med., New Orleans, La.] *Yale J. Biol. & Med.,* **17**:311-318. 1944.

In mice, gastric mucin did not favor growth (upon transplantation) of a lymphosarcoma. Tumor growth did not follow the subcutaneous injection of these neoplastic lymphocytes suspended in a solution of mucin in normal saline (31 animals). Tumor growth attended intraperitoneal injection of the same material in 21 of 25 animals. Transplantation (subcutaneous and intraperitoneal) was always successful when the tumor was injected as a suspension in normal saline or directly grafted as a small piece of tissue. Apparently, the mucin inhibited growth of the transplanted tumor by augmenting the local "foreign body" and inflammatory processes to such a degree that the neoplastic cells were destroyed. The limited areas involved in the subcutaneous sites seemed to favor these processes which destroyed the tumor cells. In the peritoneal cavity the reaction was not sufficient to prevent effectively the growth of the transplanted tumor cells. This was perhaps due not only to the greater size of the area involved but also to the apparently mild inflammatory response of the serosal lining and of other possible phagocytic elements of this site to the mucin-tumor mixtures.—Author's summary. (J. L. M.)

The Importance of Protein Intake in Cancer. CONNELL, H. C. [Hendry Connell Research Foundation, Kingston, Canada] *Canad. M. A. J.,* **52**:64-68. 1945.

In 40 mice placed on a low protein diet (10% casein) 10 days after implantation with Belough sarcoma, the tumor grew less than half as rapidly as it did in 40 control mice kept on a normal diet (Purina dog chow). The tumors disappeared spontaneously in 5 of the animals on the low protein diet, and in only 1 of the controls. The 10% protein level in the diet of the experimental group of mice was just sufficient to maintain positive nitrogen balance and did not cause serious loss of weight or other untoward symptoms. The results suggest that, by slowing the growth of the tumors, the low protein diet favors natural body defenses against them.—M. H. P.

The Influence of "Folic Acid" on Spontaneous Breast Cancers in Mice. LEUCHTENBERGER, R., LEUCHTENBERGER, C., LASZLO, D., and LEWISOHN, R. [Mt. Sinai Hosp., New York, N. Y.] *Science,* **101**:46. 1945.

Daily intravenous injections of 5 μgm. of *Lactobacillus casei* factor ("folic acid") were given to 89 mammary cancer-bearing mice of the A, Bagg, and Rockland strains, over periods of 4 to 6 weeks. In 38 of these animals the cancers regressed completely, whereas there were no regressions of the mammary cancers in 60 control mice. Only 1 new tumor appeared during the experiment among the folic acid-injected mice, compared with 14 new ones among the controls.—R. B.

The Effect of Insoluble Radiophosphorus (Chromium Phosphate) When Applied Interstitially in the Treatment of Adenocarcinoma of the Mamma in Mice. ALLEN, H., HEMPELMANN, L. H., JR., and WOMACK, N. A. [Washington Univ. Sch. of Med., Mallinckrodt Inst. of Radiol., and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Cancer Research,* **5**:239-246. 1945.

When the radioactive isotope of phosphorus is injected into the body in the form of an isotonic solution of disodium phosphate, it may be recovered in large amounts in the bone and bone marrow. Because of the radiosensitivity of the cells of the bone marrow as compared with that of most carcinomas, destructive changes are usually noted in the marrow cells before damage can be detected in the cells of such common cancers when treated experimentally by the oral or intravenous injection of radioactive disodium phosphate. To obviate this difficulty, radioactive chromium phosphate, an insoluble salt, was injected in saline suspension around the periphery of transplanted spontaneous mammary adenocarcinomas in the C57 strain of mice, with resulting regression of the tumor. Most of the radioactive material apparently remains at the site of injection, although a small portion of it is carried to the regional lymph nodes, where definite evidence of its action can be noted. There was no microscopic evidence of distant parenchymatous change. These tumors could be completely and safely destroyed by this method of interstitial radiation, except when the neoplasms were larger than one-fifth of the body weight of the animal. In such animals fatal toxemia often results from the absorption of broken-down tumor tissue.—Authors' abstract.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Radiation

Low-Voltage Contact Irradiation Therapy: Further Experience. Goin, L. S. [Los Angeles, Calif.] *Radiology*, **42**:241-245. 1944.

The results obtained with contact radiation therapy in certain benign and malignant lesions have been very good. Papillomas, keratoses, certain types of keloids, hemangiomas, carcinomas of the bladder, epitheliomas of the lip, intraoral cancer, and epitheliomas of the skin have responded to this type of treatment. Large doses can be given safely because of the high surface dose and low depth dose. The method has proved unsuccessful in vaginal carcinoma.—R. E. S.

Depth Dose Measurements for 100-, 120-, and 135-kv. Roentgen Rays. Braestrup, C. B. [New York City Dept. of Hosps., New York, N. Y.] *Radiology*, **42**:258-272. 1944.

Depth dose tables for low and intermediate voltage therapy are given for various technics. The author feels that half-value layers of 1.0, 2.0, 4.0, and 8.0 mm. Al and target-skin distances of 15 and 30 cm. should suffice for treatment in most instances.—R. E. S.

Million-Volt Isodose Curves for Angulated Beams. Reinhard, M. C., and Goltz, H. L. [State Inst. for Study of Malig. Dis., Buffalo, N. Y.] *Radiology*, **42**:591-594. 1944.

Isodose curves for various angulations of the million-volt x-ray beam are given.—R. E. S.

Nomographic Aids in Calculating Radium Dosage for Plane and Point Sources. Wolf, B. [Mt. Sinai Hosp., New York, N. Y.] *Radiology*, **42**:368-374. 1944.

Nomograms are provided from which dosage can be calculated for radium sources distributed on square or circular areas and for point radium sources, and use of these simple charts is explained.—R. E. S.

Dosage Table for Linear Radium Sources. Quimby, E. H. [Columbia Univ. Coll. of Physicians and Surgeons, New York, N. Y.] *Radiology*, **43**:572-577. 1944.

Dosage tables for linear sources of radium expressed in gamma roentgens per 100 mgm. hr. are presented and their use is illustrated by several examples.—R. E. S.

Direct Current Combined with X-Ray Therapy: Case of Kaposi Sarcoma Thus Treated. Sigel, H. [Univ. of Cincinnati Coll. of Med., Cincinnati, Ohio] *Radiology*, **43**:386-390. 1944.

A case of Kaposi's sarcoma. One lesion treated with combined x-ray therapy and local galvanic current showed better clinical response than a control lesion in the same patient, treated by x-ray alone.—R. E. S.

Calcified Spinal Meningioma. Osgood, E. C., Arnett, J. H., and Lewy, F. H. [Hosp. of Univ. of Pennsylvania, Episcopal Hosp., and Univ. of Pennsylvania, Philadelphia, Pa.] *Radiology*, **43**:62-64. 1944.

A case of meningioma of the spinal cord in the cervical region is reported in which the diagnosis could be made by means of roentgenograms because of intraspinal calcification.—R. E. S.

Tissue Dose Estimation in Combined Roentgen and Radium Therapy for Carcinoma of the Uterine Cervix. Howes, W. E. [Brooklyn, N. Y.] *New York State J. Med.*, **44**:1563-1568. 1944.

The technic of tissue dosage estimation in combined roentgen and radium therapy for carcinoma of the uterine cervix as carried out at the Brooklyn Cancer Institute is described. Examples are given.—J. L. M.

The Ureter and Its Involvement in Pelvic Irradiation. Mansur, E. E. [Jefferson City, Mo.] *Radiology*, **43**: 147-154. 1944.

The frequent damage to the ureters as an aftermath of heavy radium or roentgen irradiation of the cervix is emphasized.—R. E. S.

Roentgen Therapy for Bronchiogenic Carcinoma. Leddy, E. T. [Mayo Clin., Rochester, Minn.] *Radiology*, **41**: 249-255. 1943.

The author reviews the literature on radiation treatment of bronchogenic carcinoma and concludes that radiation has a definite place in the palliation of this disease. To avoid the complications of overtreatment simple cross-firing with moderate doses of x-rays is recommended.—R. E. S.

The Roentgen Diagnosis of Biliary Tract Tumors. Brown, S., McCarthy, J. E., and Fine, A. [Cincinnati, Ohio] *Radiology*, **41**:459-463. 1943.

For roentgen aid in diagnosis of tumors of the liver, gall bladder, and extrabiliary ducts, chest plate and fluoroscopy, flat plate of the abdomen, and films of the gastrointestinal tract are indicated. Liver enlargement displaces the stomach and duodenum to the left and backward, while disease of the gall bladder displaces them only to the left. A defect in the region of the superior angle of the duodenum may occur as the result of pressure from the neck of the gall bladder or the cystic or common duct dilated by a neoplasm or stone.—R. E. S.

Radiation Therapy in Carcinoma of the Rectum and Sigmoid. Pohle, E. A., McAneny, J. B., and Lovell, B. K. [State of Wisconsin Gen. Hosp., Madison, Wis.] *Radiology*, **41**:225-232. 1943.

An experimental study, on a small number of dogs, of the effect of radiation on rectal mucosa led to the conclusion that doses exceeding 900 r (air dose) produced ulceration and perforation of the rectum, and death of the animals. An analysis of 195 cases of carcinoma of the rectum in man showed that x-ray has a definite place in the palliative treatment of this disease. Treatment was given: (1) in cases of primary inoperable tumors, sometimes with radon implantation as well as x-ray; (2) in cases in which the tumor became operable after a cycle of radiation; (3) postoperatively; (4) for recurrent disease. The value of postoperative therapy has not been incontrovertibly determined.—R. E. S.

Multiple Myeloma First Discovered in the Mandible. Wolff, E., and Nolan, L. E. [Laird Memorial Hosp., Montgomery, W. Va.] *Radiology*, **42**:76-78. 1944.

Report of a case treated by x-ray, with temporary benefit. Physicians and dentists are urged to take specimens

for biopsy of every noninflammatory lesion of the gums, alveolar processes, and jaw bones.—R. E. S.

Use of the Basal Metabolic Rate in the Management of Radiotherapy for Leukemia. Uhlmann, E. M., and Goldner, M. [Michael Reese Hosp., and Univ. of Chicago, Chicago, Ill.] *Radiology,* **42**:165-174. 1944.

A study of the relationship between basal metabolism and leukemia indicates that the course of the disease can be followed by the basal metabolic rate. When an exacerbation is approaching, the basal metabolic rate is elevated before any other manifestations appear, and the rate drops when effective treatment has been instituted. Very small amounts of x-ray are usually all that are necessary to control the disease, and if the metabolic rate is used as an indicator, the patient may be able to tolerate treatment for a longer period, and life may be prolonged. Illustrative case histories are given.—R. E. S.

Leukemia in Radiologists. March, H. C. [Philadelphia, Pa.] *Radiology,* **43**:275-278. 1944.

In the United States during the past 15 years, leukemia was responsible for 8 (4.57%) of 175 deaths of radiologists, and for 221 (0.44%) of 50,160 deaths of physicians who were not radiologists. Besides the 8 cases in the United States, there are 23 cases, reported in the world literature, of leukemia in persons exposed for a long period to radiation.—M. H. P.

Industrial Radiation Hazards. Braestrup, C. B. [New York, N. Y.] *Radiology,* **43**:286-292. 1944.

The necessary precautions against radiation exposure in industrial plants are discussed with protection charts given for various voltages, milliampere minutes, and distances.—R. E. S.

The Development of Centralised Radon Services in Australia. Eddy, C. E. [Univ. of Melbourne, Melbourne, Australia] *Radiology,* **43**:155-169. 1944.

Australia has 5 radon-producing centers from which radon is issued to smaller qualified hospitals and private practitioners. The system has many advantages, which are discussed in detail.—R. E. S.

Nervous System

Intracranial Meningiomas. Kazan, A. T., Weller, D., and Jaramillo, J. G. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.,* **11**:105-128; 169-184. 1944.

Tumors derived from the meningeal coverings of the brain are among the more common types of brain tumor and lend themselves to successful surgical removal. A review of the current workable concepts relative to gross anatomical, histological, and clinical features of these tumors serves as the main theme of this comprehensive paper. The illustrative cases include 74 verified by postmortem examination and 90 proved by biopsy. The incidence of the tumor, anatomical distribution, clinical and roentgenologic characteristics, cerebrospinal fluid, and electro-encephalographic findings in these cases are discussed. The histologic classification of Globus and the clinical classification of Cushing together offer the clearest, most comprehensive, and most workable basis for the recognition, localization, and final diagnosis of these tumors.—A. Cnl.

Relation of Abnormal Collections of Cells in Posterior Medullary Velum of Cerebellum to Origin of Medulloblastoma. Raaf, J., and Kernohan, J. W. [Univ. of Oregon Med. Sch., Portland, Ore., and Mayo Clin., Rochester, Minn.] *Arch. Neurol. & Psychiat.,* **52**:163-169. 1944.

The material examined in this study consisted of the cerebellums of 161 embryos, fetuses, and infants; the youngest embryo was one of 8 weeks, and the oldest child was 5 years of age. Of 104 cerebellums in which the posterior medullary velum was examined microscopically, 23 contained abnormal collections of cells. These cells were morphologically similar to cells of the medulloblastoma. Since the site of abnormal collections of cells and the point of origin of medulloblastomas were observed to be the region formerly occupied by the germinal bud, the authors think the medulloblastoma arises frequently, if not exclusively, from cell rests that occur in this region.—M. E. H.

Meningioma of the Velum Interpositum. Wycis, H. T. [Temple Univ. Hosp. and Sch. of Med., Philadelphia, Pa.] *Arch. Neurol. & Psychiat.,* **52**:534-537. 1944.

A case report. The possible success of the surgical removal of a psammomatous meningioma was marred by fatal hemorrhage from a tracheotomy wound.—M. E. H.

Clinical and Pathologic Features of Gliomas of the Spinal Cord. Shenkin, H. A., and Alpers, B. J. [Jefferson Med. Coll., and Univ. of Pennsylvania, Philadelphia, Pa.] *Arch. Neurol. & Psychiat.,* **52**:87-105. 1944.

A review of 27 verified cases of intramedullary gliomas of the spinal cord. The clinical features have been correlated with the pathologic findings in an attempt to clarify their diagnostic characteristics.—M. E. H.

Neuroepithelioma of the Radial Nerve with a Study of Its Behaviour *in vitro*. Stout, A. P., and Murray, M. R. [Columbia Univ. Coll. of Physicians and Surgeons, and Presbyterian Hosp., New York, N. Y.] *Rev. canad. de biol.,* **1**:651-659. 1942.

Report of a tumor of the radial nerve in a 35 year old male. The tumor expanded the nerve at the bend of the elbow and extended cephalad within its sheath. One year after amputation of the arm there was x-ray evidence of bilateral lung metastases. Histologically the tumor was composed of undifferentiated cells. Mitoses were present. Cultivated *in vitro,* the tumor cells exhibited the growth characteristics of early undifferentiated embryonic epithelium. Cultivation was stopped after 25 days as no changes in morphology or behavior of the cultures occurred. The authors interpret this explant as neuroepithelium and classify the tumor as neuroepithelioma.—C. A.

Neurinoma of the Mental Nerve. Amyot, B. E. *U. S. Nav. M. Bull.,* **43**:125-126. 1944.

Report of a case in which a tumor the size of a walnut arose at the site of a blow on the chin.—C. W.

General Neurofibromatosis (von Recklinghausen's Disease) with Local Sarcomatous Change and Metastasis to Regional Lymph Nodes. Wachstein, M., and Wolf, E. [Beth Israel Hosp., Passaic, N. J.] *Arch. Path.,* **37**:331-333. 1944.

A case report.—J. G. K.

Brief Clinical Notes on Two Cases of Retroperitoneal Schwannoma. STALLWORTHY, J. *Proc. Roy. Soc. Med.*, **37**:439-440. 1944.

The author points out that these tumors are usually single and nonmalignant, and if adequately removed do not recur. They must be differentiated from the neurofibroma of von Recklinghausen's disease. They produce symptoms by pressure effects and by constitutional reaction secondary to degenerative changes. The histological picture is that of capricious degeneration, in which zones of intercellular edema are present *pari passu* with the characteristic nuclear palisades.—L. W. P.

BREAST

Das Ergebnis der Behandlung des Cancer mammae in der Chirurgischen Klinik in Uppsala in den Jahren 1914-1933. [Results of Treatment of Mammary Cancer in the Surgical Clinic in Uppsala during 1914-1933.] LINDQUIST, G. [Surg. Clin., Uppsala Univ., Uppsala, Sweden] *Acta chir. Scandinav.*, **86**:349-358. 1942.

Of 78 and 156 women operated upon for cancer of the breast in 1914 to 1923 and 1924 to 1933 respectively, 18% and 34% were free from recurrence 5 years after operation. The difference between the results for the 2 decades is not statistically significant. During 1929 to 1933, when radiological therapy was generally employed as an adjuvant to surgery, the results were not better than during 1924 to 1928. The age of the patients had no apparent bearing on the outcome of surgery.—M. H. P.

Malignant Tumor of Breast. MOTLEY, E. G., and HARWOOD, D. A. [Santa Ana, Calif.] *California & West. Med.*, **59**:123. 1943.

Description of a 22 lb. tumor with no pathological report to support the diagnosis "malignant."—W. A. B.

Discussion on Advanced Cases of Carcinoma of the Breast Treated by Stilboestrol. ELLIS, F., ET AL. *Proc. Roy. Soc. Med.*, **37**:731-736. 1944.

ELLIS, F. Of 21 patients, ages 35 to 81, who received about 15 mgm. of stilbestrol daily without radiation treatment, 3 were intolerant, 4 (mean age 64.2 years) improved.

ADAMS, S. B. A woman of 82 had nodular recurrences that disappeared under treatment (5 mgm. 3 times a day) lasting 2 months. A dose of 1 mgm. daily may produce noticeable improvement.

BLOMFIELD, G. W. No striking results were obtained in 10 cases (2 to 15 mgm. daily) and no change in growth rate of the tumor was noted in 5 of these cases in which accurate measurement was possible.

HADDOW, A. Thirteen patients received a mean total dose of 350 mgm. in over 11 weeks. Details of these cases are given elsewhere (*Brit. M. J.*, **ii**:393-398. 1944; abstr. in *Cancer Research*, **5**:128. 1945). Nine of the patients seemed to be quite unaffected; in 3 there was temporary retardation of tumor growth or regression; 1, of whom photographs are given, showed considerable regression of the primary tumor and of deposits in the axillary lymph nodes. The mean age of the 4 patients showing any favorable response was 62, and that of the remaining 9 patients was 48.

LEVITT, W. M. Improvement was maintained for 4 months in 1 patient, aged 69; 10 other patients, all under 60, did not respond. The dosage was 2.5 to 5 mgm. 3 times a day.

MCWHIRTER, R. "Very definite improvement" occurred in 6 (youngest 52, mean age 62 years) of 37 patients, but was not maintained in 2 of these. The dosage was 5 mgm. twice a day.

THURGAR, C. J. L. Ten consecutive patients received not less than 10 mgm. daily. Favorable responses occurred immediately if at all. In 5 cases no effect was observed; in 5 patients there was some improvement, 1 of these (aged 58) showing a "rapid and spectacular response," and 2 showing continued and 2, transient improvement. No patient under 58 improved, and the majority of persons who responded were well over 60. Some metastases may regress while others enlarge or new ones appear. Cancer of the breast may show spontaneous remissions or regressions.

WALKER, J. Z. Ten patients received 1.2 to 10 mgm. thrice daily; the average age of 3 showing considerable improvement was 67.3 years. Five patients were not benefited and in 2 the effect was adverse.

WINDEYER, B. W. Of 10 very advanced cases, 3 showed "demonstrable improvement."

DODDS, E. C. Hexestrol and dienestrol were suggested for trial as possible substitutes for stilbestrol.

PATERSON, E. Stilbestrol (average 5.8 mgm. daily) was given to 13, and triphenylchloroethylene (average 3 gm. daily) to 23 patients. Regression under either drug was most distinct in "the established primary mass, whether this was the original breast tumor or recurrent nodules in the chest wall. Bone metastases were also sensitive either regressing or ceasing to give pain (4 cases)." Metastases in lymph nodes were on the whole less sensitive. Regression appeared if at all within a few weeks of the commencement of treatment. The more cellular tumors responded best. Neither compound proved as effective as palliative x-ray therapy.

Symptoms attributable to stilbestrol that were noted by various contributors to the discussion were nausea, vomiting, tenderness of breasts, swelling and pigmentation of the areola, resumption of menstruation (so severe in 1 case as to necessitate a blood transfusion), and edema of the ankles.

Those observers who reported favorable results were unanimous in finding that these occur among the older women: improvement was noted in 27 of 68 women aged 60 years or more, and in 14 of 100 less than 60, according to combined tabulated data. Dr. Haddow in his contribution discussed the lines of research that must be pursued to throw light on this matter.—E. L. K.

Lesions of the Male Breast. EICHERT, H. *U. S. Nav. M. Bull.*, **42**:1350-1356. 1944.

Emphasis is laid upon the 2 most common conditions that might be encountered—diffuse hyperplasia and carcinoma. Their clinical and pathological features and treatment are presented.—C. W.

Male Genital Tract

Carcinoma of the Prostate Gland. An Analysis of 88 Fatal Cases from Charity Hospital of Louisiana at New Orleans, with a Special Note on Newer Methods of Therapy. Kahle, P. J., and Beacham, H. T. [Sch. of Med., Louisiana State Univ., New Orleans, La.] *Urol. & Cutan. Rev.*, **48**:1-10. 1944.

From an analysis of 88 cases of carcinoma of the prostate gland, it was concluded that castration and stilbestrol therapy are very valuable but not curative. The commonest causes of death in this series were carcinomatosis, urinary tract infection, and degenerative diseases associated with age. Among 46 autopsies, metastases were found in 22 instances, and malignant tumors elsewhere in the body in 7. It is evident that diagnosis is difficult since 22 carcinomas were discovered only at autopsy, and 4 patients had had transurethral resections for tumors diagnosed before death as benign.—V. F. M.

Androgen Control in Carcinoma of the Prostate. Bowler, J. P., and Pedley, S. F. [Dartmouth Med. Sch., and Mary Hitchcock Memorial Hosp., Hanover, N. H.] *New England J. Med.*, **230**:501-505. 1944.

Short review paper including discussion of rationale and surgical technic of orchidectomy, with case reports selected from 22 cases of carcinoma of the prostate treated by the authors during 1942. The results in all cases are not tabulated, but interesting features of some of them are discussed to illustrate the following points: (1) negative roentgen ray findings for lumbar spine and pelvis, and absence of pain do not exclude the presence of metastases, (2) serum acid phosphatase level is of great significance, but changes do not in all instances correlate well with the clinical course, (3) relapse may occur after cases have apparently been brought under control, or improvement may continue after orchidectomy, with regression of metastases and recalcification of involved bone. In general, the authors conclude that no other method of therapy for far advanced cases offers relief for so long or so completely as do the procedures described. The relief and comfort obtained in cases of remission following routine deep x-ray therapy have been striking.—C. W.

Plasma Acid Phosphatase in Carcinoma of the Prostate and the Effect of Treatment with Stilboestrol. Watkinson, J. M., Delory, G. E., King, E. J., and Haddow, A. [Roy. Cancer Hosp. (Free), and Brit. Postgrad. Med. Sch., London, England] *Brit. M. J.*, **ii**:492-495. 1944.

The authors summarize very briefly the literature relating to acid phosphatase and the normal and cancerous prostate and refer to the review by Haddow (*Brit. J. Radiol.*, **16**:193. 1943) of this subject. The estimations of acid and alkaline phosphatase were carried out by the method of King *et al.* (*Lancet*, **242**:207. 1942). The range in the plasma of normal persons was 1 to 5 units of acid, 3 to 10 units of alkaline phosphatase per 100 ml. In 50 cases of anemia, heart disease, cholecystitis, catarrhal jaundice and hepatitis, obstructive jaundice, and primary carcinoma of the stomach, and in 12 cases of enlarged prostate, the acid phosphatase values were within this normal range, while in 8 cases of prostatic carcinoma without bony metastases the range was 1 to 6, and in 7 cases with such metastases the range was 6 to 19 units. The alkaline phosphatase, which reached very high levels in obstructive jaundice (10 to 163 units), showed a similar difference between cases of cancer of the prostate without (4 to 11 units) and with (7 to 87 units) bony metastases. Human seminal fluid contained amounts of acid phosphatase about 100,000 times greater than those present in plasma; fluid from 2 cases of eunuchoidism showed about 1/100 of the normal concentration.

In a further series of 10 patients with prostatic carcinoma, 6 had abnormally high plasma acid phosphatase values (up to 41 units) and in 5 of these there was radiographic evidence of metastases in bone. The plasma acid phosphatase was increased also in some cases of Paget's disease. When a patient with prostatic carcinoma is treated with stilbestrol (intramuscular), the plasma acid phosphatase, if raised previously, falls abruptly to the normal level, while the alkaline phosphatase shows an increase followed by a gradual fall. Details are recorded of 10 patients treated with stilbestrol given usually at first by the intramuscular route (dose: 34 mgm. in 28 days to 525 in 21) and then orally (1 to 5 mgm. once, twice, or thrice daily). Intramuscular injection seems to have no advantage except that the amount introduced is known exactly. In 4 of the 10 cases some regression of the primary tumor took place, and in 3 cases there was a slow diminution during treatment in size of lymph nodes bearing secondary deposits. Metastases in bone may show by x-ray examination an increase in size and number, or increase in density. Nine of the 10 patients showed some improvement in general condition (relief of pain, lesser frequency of micturition, increase in hemoglobin, gain in weight), but the authors recognize that rest and administration of iron may have contributed to these changes.—E. L. K.

Sarcoma of the Prostate Gland. Miller, S., and Barshay, B. [Vet. Admin., Legion, Tex.] *M. Bull. Vet. Admin.*, **20**:417-421. 1944.

A general review of the literature accompanies the case report.—M. E. H.